BIONOMICS OF THE PRIMARY MALARIA VECTOR, *ANOPHELES PSEUDOPUNCTIPENNIS*, IN THE TAPACHULA FOOTHILL AREA OF SOUTHERN MEXICO

1992

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ABSTRACT

Bionomics of the primary malaria vector, *Anopheles pseudopunctipennis,* in the Tapachula foothills area of southern Mexico

Ildefonso Fernández-Salas, Doctor of Philosophy, 1992.

Dissertation directed by: Donald R. Roberts, Professor. Department of Preventive Medicine and Biometrics, and Mario Henry Rodriguez, Adjunct Associate Professor

Malaria, the more important vector-borne disease in Mexico, is transmitted predominantly by *Anopheles pseudopunctipennis* in roughly two-thirds of the malarious areas of Mexico. Included in this report are the results of studies to clearly define the relationships of humans to *An. pseudopunctipennis* mosquitoes in foothill areas near Tapachula, Chiapas, Mexico.

Mark-recapture methods were used in studies of the gonotropic cycle. A 3-day cycle was documented for wild-caught, marked and released populations of mixed physiological age. A 4-day cycle was documented for marked and released females that were laboratory-reared. The Human Blood Index (HBI), which is the percentage of human blood meals in engorged mosquitoes, was greater than 30% in pooled samples from all study sites. A shift from feeding on humans to feeding on other animals occurred with outdoor host-seeking populations after DDT was sprayed on house walls. Approximately 60% of all nulliparous females required a second blood meal to complete their first gonotrophic cycle. This phenomenon greatly increases the capacity of this vector to acquire and transmit malaria.

During the wet season most larvae were collected from largely temporary habitats, such as seepage spring, rainwater pool and pond habitats containing filamentous algae. Coincidentally, the largest numbers of mosquitoes coming to human hosts were in those villages located closest to the river. Transects were employed to quantify the seasonal dynamics of *An. pseudopunctipennis* larvae. The primary factor was availability of aquatic habitats with filamentous algae. In hierarchical order, habitat availability is regulated by the

quantity and turbulence of water flow in the river. Finally, water flow is regulated by rainfall in the mountain and foothill areas.

The finding of the new variant *P. vivax* (PV 247) antigen in wild-caught specimens of *An. pseudopunctipennis* further extended the geographical range of this Asian strain of *P. vivax* malaria.

Peak malaria transmission rates and peak densities of *An. pseudopunctipennis* mosquitoes occurred during the dry season. The results emphasize the importance of *An. pseudopunctipennis* as a malaria vector in Mexico, as well as the need to link entomological and other types of data to understand better the epidemiology of malaria in a particular environment.

Bionomics of the primary malaria vector, *Anopheles pseudopunctipennis*, in the Tapachula foothills area of southern Mexico

by

Ildefonso Fernández-Salas

Dissertation submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy 1992

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I cannot overlook those persons near to me, who provided that vital "logistic"-emotional support throughout all my student life: my wife Martha and my daughter Ana Cristina, who arrived in our lives in 1990.

All men are like grass,
and all their glory is like the
flowers of the field;
the grass whiters and the flowers fall,
but the word of the Lord stands
forever

1 Peter 1: 24-25.

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Malaria continues to be a major problem of many tropical developing countries, and in recent years malaria has reappeared in many areas that were freed of the disease in the 1960s (Bruce-Chwatt, 1985). More than 2,073 million people, or 40% of the world's population in approximately 100 countries and territories, continue to be at risk of contracting malaria. Of a total world population of 5.16 billion people, 1.4 billion (27%) live in regions where malaria never occurred or where it disappeared without intervention. Another 1.65 billion (32%) live in regions where malaria has been eradicated through control programs and where it has not recrudesced. However, malaria has recrudesced in many areas where it had been greatly reduced or even eradicated. Roughly 1.62 billion people live where malaria has returned as a great public health problem. In some populations, malaria is a major problem because of significant ecological and sociological changes, and this seems to be true for about 1% of the world's population. Finally, 490 million people live in areas where malaria endemicity remains practically unchanged, where there may be intense transmission, and where malarial control programs have never been fully implemented. These unprotected populations are found mainly in tropical Africa (World Health Organization, 1991a, 1991b). The number of cases officially reported to WHO has been only about five million per annum for the past three years (excluding figures from the WHO African Region). More realistic estimates put the numbers of clinical cases of malaria per year at 110 million, of which 90 million are from tropical Africa. Furthermore, the number of deaths due to malaria is estimated to be approximately one million a year. In 1989, 5.2 million malaria cases were reported to the WHO, excluding Africa, and 95% were from just 25 countries. Half of all these cases were registered in just 2 countries; *viz.*, India (39%) and Brazil (11%) (World Health Organization, 1991a, 1991b).

In the Americas, an estimated 278 million people were living in malarious areas in 1990. Morbidity from malaria was 149.67 per 100,000 people, while in 1974, morbidity was only 49.37 per 100,000 inhabitants. Furthermore, more than one million cases have been registered in the Region during each of the last four years (Pan American Health Organization, 1991). These statistics are indicative of destabilized and deteriorating conditions that will adversely impact the governments, societies and economies of the whole region. Overall, 53.5% of all malaria cases were reported from Brazil, and 24.3% from the countries in the Andean Region; *viz.*, Bolivia, Colombia, Ecuador, Perú and Venezuela. Mexico accounted for 4.2% of all cases in the Americas in 1990 (Pan American

Health Organization, 1991).

As recently as 1989, Mexico accounted for approximately 10% of all malaria cases in the Americas (Rodriguez and Loyola, 1989). The increased numbers of malaria cases in Mexico began in the late 1970s, with a peak of 133,698 cases occurring in 1985 (Fig. 1). This trend was reversed in 1986 through application of integrated control methods and a stratification of attack measures by the Mexican Secretariate of Health. Consequently there were 56% fewer cases of malaria in Mexico in 1990 than in 1989 (Dirección General de Epidemiología-Secretaría de Salud, 1991). Similarly, 16,102 villages reported malaria cases in 1989, while there were 34% fewer villages reporting malaria cases in 1990 (Direccion General de Epidemiología-Secretaría de Salud, 1991). More than 99% of all cases of malaria in Mexico are due to *P. vivax* infections, and most cases occur in just five states; *viz.*, Guerrero, Oaxaca, Chiapas, Michoacan and Sinaloa. These states are located along the Pacific Coast, which is the most endemic region in the country (Rodriguez and Loyola, 1989).

As in other countries, the recrudescence of malaria in Mexico was due to developmental projects in malarious areas, to influxes of migrant workers, poorly constructed houses, insecticide resistance, financial constraints in the government control program, and lack of personnel trained in malaria control (Rodriguez and Loyola, 1989)

A total of 25 anopheline mosquitoes are known to occur in Mexico (Vargas and Martinez-Palacios, 1955). Included in the list of actual and potential malaria vectors is *Anopheles albimanus* Wiedemann, *An. pseudopunctipennis* Theobald, *An. vestitipennis* Dyar and Shannon and *An. darlingi* Root. Based on their geographical distribution, *An. albimanus* and *An. pseudopunctipennis* are considered to be the primary vectors throughout most of Mexico. The former is found on the plains of the Pacific Coast of southern Mexico, the Mexican Gulf and the Yucatan Peninsula region. Populations of *An. pseudopunctipennis* are found throughout most of the Mexican territory at higher elevations (0 to 2000 m). This species seems to be more abundant than *An. albimanus* in roughly two-thirds of the malarious area (Rodriguez and Loyola, 1989).

Anopheles pseudopunctipennis was originally described by Theobald in 1901 from the Caribbean Island of Grenada. It is native to the Neartic and Neotropical regions, where it is found in the southern half of the United States, most of Mexico, throughout Central America and in the Andean Regions. More specifically, this vector occurs in the South American countries of Venezuela, Colombia, Ecuador, Peru, Bolivia, Chile and the Northern half of Argentina (Fig. 2). In the Caribbean, it has been found in Trinidad and Grenada, and recently it was collected in Haiti (Molez, *et al.*, 1987). Taxonomists have described five subspecies and one variety. The species-type is designated as *Anopheles*

pseudopunctipennis pseudopunctipennis (Knight and Stone, 1977). The subspecies are distributed in four countries of South America, *levicastilloi* and *rivadeinerai* in Ecuador; *neghmei* and *noei* in Chile; and *patersoni* in Argentina. The single variety, *bifoliata*, is reported from Colombia (Fig. 2). *Anopheles pseudopunctipennis* is thought to represent a complex of sibling species within its extensive geographical range (Baker, *et al.*, 1965).

The role of this anopheline as a malaria vector was first reported from Panamá by Darling (1910). He noted that 14.8% (4/27) of *An. pseudopunctipennis* mosquitoes that fed on a patient with vivax malaria became infected. Salivary gland infections in wild-caught specimens have been documented in Argentina, where 0.7 to 2.1% were infected (Davis, 1927), and 1.8% were infected in Perú (Hayes, *et al.*, 1987); and 2.2% of the midguts were infected in Temixco, in Central Mexico (Vargas, 1938). As much as 3.16% of wild-caught females from Sinaloa, in Northwestern Pacific Mexico were found to have *P. vivax* CS-proteins (Loyola, *et al.*, 1991). According to Aitken (1945) and Boyd (1949) this species has been incriminated as a vector of malaria in Guatemala, Salvador, Honduras, Nicaragua, Panama, Venezuela, Ecuador and Bolivia. *Anopheles pseudopunctipennis* appears to be responsible for most malaria transmitted at higher elevations (Hackett, 1945).

This species seems to prefer to feed on humans and to rest inside dwellings. In Argentina, Shannon and Davis (1928) found that 50% of 580 females contained human blood; Acosta (1960) in Perú identified human blood in 80.6% of indoor resting females; and in Central Mexico, Vargas (1938) found that 67.6% of 244 females contained human blood. A habit of resting inside of unsprayed houses was documented for this species in Acatlipa, a small rural village in Central Mexico (Gahan, *et al.*, 1947). Bordas and Downs (1951) defined a partial endophilic behavior for *An. pseudopunctipennis* in unsprayed dwellings by finding that 26.3% of the females resting on housewalls during the day were in an advanced stage of egg development. In other words, they demonstrated that an unknown percentage of the total population of females rested inside the house during part or all of the 48-72 hours required for the gonotrophic cycle; this distribution of resting females represents, by definition, some degree of endophilic behavior. The studies by Bordas and Downs were conducted in central Mexico.

The endophilic behavior of *An. pseudopunctipennis* implies that this species is a good target for control by spraying housewalls with insecticides. However, physiological resistance to DDT has been demonstrated in México and Perú, which means that more expensive compounds might be needed to interrupt man-vector contact (World Health Organization, 1980). Loyola, *et al.* (1991) demonstrated a higher excito-repellency effect of *An. pseudopunctipennis* for DDT than for Bendiocarb. Loyola and coworkers (1990)

demonstrated that both insecticides induced some avoidance behavior; however, the proportion of indoor resting females with human blood was higher in Bendiocarb-sprayed houses than in houses sprayed with DDT. In earlier studies, DDT avoidance by *An. pseudopunctipennis* was documented for populations in Central Mexico by Martinez-Palacios and De Zulueta (1964).

Larvae of *An. pseudopunctipennis* are found in sunlit pools containing very clear water. Such pools often develop in margins of rivers as the flow of water decreases during the dry season. These optimal riverine environments are most frequently encountered in hilly or mountainous regions. These relationships account, in part, for the observation that *An. pseudopunctipennis* populations are generally more abundant at altitudes above 200 m (Hackett, 1945). Another characteristic of *An. pseudopunctipennis* breeding sites is the rather universal presence of dense mats of filamentous algae. The association of larval populations with filamentous algae is consistent throughout the species' extensive geographical distribution from North to South America (Shannon and Davis, 1927; Hoffman and Samano, 1938). Larvae disappear when algal mats are either flushed away by heavy rains or when pools dry out during a prolonged dry season (Shannon, 1930).

Although *An. pseudopunctipennis* is an important vector of malaria throughout much of Central and South America, it has been the subject of relatively few studies in recent years. A search of the literature on *An. pseudopunctipennis* resulted in a compilation of only 305 articles. A similar search for literature on *An. albimanus* resulted in a compilation of roughly 2,000 citations (Frederickson, 1988).

Only 98 (32.1%) of the 305 articles on *An. pseudopunctipennis* dealt directly with some aspect of the vector's biology. The remaining 207 references dealt with *An. pseudopunctipennis* as a species of secondary importance to some other vector species; *e.g., An. albimanus* in Mexico and Central America, or *An. nuñeztovari, An. punctimacula, An. argyritarsis* and *An. oswaldoi.* Some articles were merely regional malaria reports, field mosquito surveys or faunistic surveys. References for special topics on *An. pseudopunctipennis* biology are listed in Annexes 1-8, and the full bibliography of available literature is included in Annex 9.

Of the 98 primary articles on *An. pseudopunctipennis* biology, about 30 articles were concerned with biting behavior and the species' role as a vector of malaria (Annex 1). Nineteen papers dealt with taxonomy and systematics of egg, larval and adult stages (Annex 2). Observations on field evaluations of chemical control measures, insecticide susceptibility, physiological and behavioral resistance and related subjects were included in 14 of the 98 references (Annex 3). A total of 16 papers were concerned with geographical distribution and distribution by altitude (Annex 4). Only six papers were written about

infections of *P. vivax* and *P. falciparum* parasites in *An. pseudopunctipennis* mosquitoes (Annex 5). Studies on the immatures were reported in six papers (Annex 6), and egg biology was the subject of four articles (Annex 7). The variable results of colonization efforts were documented in four papers (Annex 8). Nearly 50% of the 305 papers reporting information on *An. pseudopunctipennis* originated from research in four countries; *viz*, Mexico (23.6%), Argentina (11.8%), Perú (5.6%) and Ecuador (4.3%)

Relatively few studies on *An. pseudopunctipennis* have been conducted in recent years. In fact, only 26 relevant articles have been published since 1980. This compares with roughly 42 articles published from 1960-1969 and again from 1970 to 1979. The remaining 196 available articles were all published before 1960; in others words, most of the scientific information on *An. pseudopunctipennis* was produced more than 30 years ago.

With the resurgence of malaria in Mexico during the 1970s and 1980s, the Malaria Research Center (Centro de Investigación de Paludismo) has served as primary national resource for malaria research. The Center is located in Tapachula, which is a city in the state of Chiapas, Mexico. Center personnel have a continuing interest in entomological studies on the biology and control of malaria vectors. Most entomological studies have been on *An. albimanus*, which is an important vector along the coastal plain. However, it has become increasingly apparent that *An. pseudopunctipennis* is of equal or greater importance in the maintenance of malaria transmission in Mexico. Consequently, there has been an increasing recognition that additional research on *An. pseudopunctipennis* biology is needed to support the goals and objectives of the National Malaria Control Program. This has occurred simply because *An. pseudopunctipennis* is more widely spread than *An. albimanus*, and because comparatively little is known about its susceptibility to malaria infections, vectorial competence, insecticide resistance, behavioral response to insecticides, adult longevity or larval biology.

The present dissertation includes observations and results of studies on four parameters of *An. pseudopunctipennis* biology, as follows:

1. Length of the gonotrophic cycle and adult longevity
2. Bloodmeal patterns and Human Blood Index (HBI)
3. Dynamics of adult populations, host-seeking behavior and malaria infection rates
4. Characteristics of breeding sites, and abundance of larvae

These topics are covered in chapters 2, 3,4 and 5, which constitute the body of the dissertation. Each chapter is written in publication format; consequently there is some

redundancy in the introduction and materials and methods sections.

The research described herein was conducted in a foothill area near Tapachula, Mexico during 1990 and 1991.

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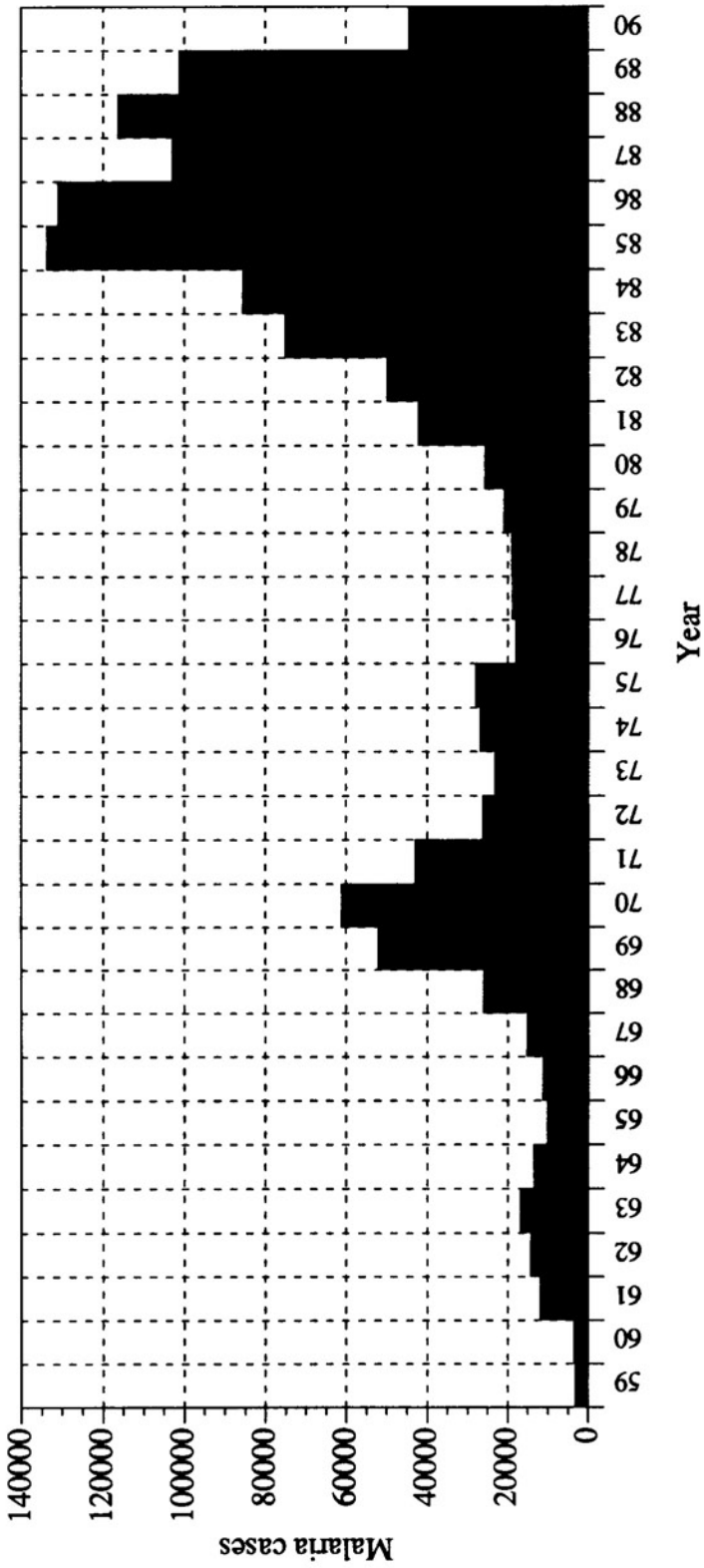
Figure 1. Malaria cases in Mexico 1959-1990



Source: Pan American Health Organization, 1991. Status of Malaria Control Programs in the Americas. XXXIX Report, Washington, DC.

Figure 2. Geographic distribution of *Anopheles pseudopunctipennis* including five subspecies and one variety.

CHAPTER 2

Gonotrophic cycle and survivorship of *Anopheles pseudopunctipennis* (Diptera: Culicidae) in the Tapachula foothills of southern Mexico

INTRODUCTION

Anopheles pseudopunctipennis Theobald, 1901 is the primary vector of malaria in nearly two-thirds of the malarious areas of Mexico (Rodriguez and Loyola, 1989; Vargas, *et al.*, 1941). This anopheline typically inhabits hilly regions, where it breeds abundantly during the dry season in isolated pools of rivers and streams. The larvae are generally found only in sun-exposed pools with dense mats of filamentous algae (*Chladophora* and *Spirogyra* spp.). This phytoecological association is very strong and is a good predictor of *An. pseudopunctipennis* breeding sites (Savage, *et al.*, 1991; Rejmanikova, *et al.*, 1991, in press).

Although *An. pseudopunctipennis* is an important vector of vivax malaria in Mexico and other countries in Central and South America, many important aspects of its biology are unknown. Little is known about the gonotrophic cycle of this vector, partly because a stenogamic colony has not been established for direct laboratory observations. Additionally, many types of field studies on adult *An. pseudopunctipennis* are difficult because of characteristically low adult population densities in the field (Chapter 4).

To better understand the biology of this important malaria vector, the field biology of *An. pseudopunctipennis* was studied from 1989 through the dry season of 1991 in the Tapachula foothills of Mexico. The number of days required for completing the gonotrophic cycle, the per cent of females that undergo pre-gravid development, and the natural parity and daily survival rates were defined in the course of this investigation.

METHODS AND MATERIALS

Description of Study Area

The Tapachula foothills are located approximately 20 km northeast of the city of Tapachula, in the state of Chiapas, Mexico. The study sites are located in the foothills of the western Pacific slope of the Sierra Madre mountain range. Most malarious villages are in the coffee-growing area at elevations of 300-700 meters. The Coatan River cuts through the foothill region, and the study sites were located along the river's margin (Fig.1). The river is bordered by hills where human settlements have been established. El Plan, a village of 340 inhabitants, is close to the study site and is the most malarious village along this stretch of the river. A steep path of 300 meters goes up from the river to El Plan.

The average annual rainfall in the study area is 3800 mm. There is a well defined wet season from May to late November, and a dry season from December to April. The mean annual temperature in the foothills is 25 ^{o}C. The cooler months are December and January, when minimum temperatures of 13-16 ^{o}C are observed between 22:00-06:00 hours. Humidity remains at 90-95% at night, even during the dry season. Prevailing winds of less than one km per hour are North to South. Studies included in this report were conducted during the dry season, and there were no heavy rains during the reporting period.

Study Plan

Engorged, wild-caught *An. pseudopunctipennis* females were employed in mark-release-recapture studies to define the length of the gonotrophic cycle. For the purposes of this study, we defined the length of the gonotrophic cycle as the number of days from one blood meal to the next.

Engorged females were collected from a horse-baited trap during the night, marked with fluorescent powder and released at sunrise. Three full-night collections were made in sequence, and females were marked with different colors on each of the three mornings. Attempts to recapture marked specimens were continued for the next 9 nights.

There were differences in average ambient temperatures from early dry season (December-January) to late dry season (March-April). Uniform studies were conducted during both periods to determine whether differences in the lengths of the gonotrophic cycles were comparable to differences in ambient temperatures.

Because wild-caught females used in these studies were of mixed physiological age (*i.e.*, nulliparous and parous) we were concerned about the influence of physiological age on determinations of time required for completion of the gonotrophic cycle. Consequently, the effect of physiological condition on gonotrophic development was assessed by rearing adults from eggs of wild-caught females, and using the reared adults in another three-day series of mark-release-recapture studies.

Additionally, a cohort of wild-caught, engorged females was monitored to document the temporal progression of oogenesis. The engorged females were held under field conditions in order to replicate light, temperature and humidity cycles that might occur in the vector's normal environment. Batches of ten females were examined at six-hour intervals throughout a 60-hour holding period. Each of the ten females per sample was examined for a determination of Sella's stage of blood digestion and then was dissected for a determination of Christophers' stage of egg development. These observations served as additional support of our findings from the mark-release-recapture studies.

Results of the mark-release-recapture studies and determinations of parity rates of field-collected specimens were used to estimate the daily survival rates of *An. pseudopunctipennis* females.

Length of the Gonotrophic Cycle

Mark-Release-Recapture Studies: Wild-Caught Females. In preliminary studies conducted in 1990, we learned that the numbers of *An. pseudopunctipennis* obtained from landing collections on humans were not sufficient for a mark-release-recapture study. However, a horse-baited trap could be used to capture large numbers of specimens, so we used this method to provide females for the mark-release-recapture studies. Each was constructed of mosquito netting and was 3 x 3 x 3 meters in size. A horse was tethered inside each active trap. Each of five traps was set in a line, 100 meters apart, and 20-50 meters from the edge of the river. The horses were secured inside the traps from 1800 h until 0500 h.

These studies were conducted by two three-man teams working in six hour shifts. The horse-baited traps were checked at two-hour intervals throughout the night, from 1900 to 0500. Freshly engorged females were found resting on the inside walls of the traps. Battery lanterns and mouth aspirators were used to collect fully-fed specimens only. The engorged females were placed in a 3.785-liter

cardboard cage, along with a small cotton pad soaked with a 10% sugar water solution.

After the last collection, at 0500 h, mosquitoes were dusted using a Pasteur pipette and a rubber bulb to spray the aerosolized powder into the cage. A different color of fluorescent dye (Lumogen Yellow® BASF, Holland, MI) was used (red, green and yellow) for each of the three consecutive nights. The marked females were released at site A (Fig. 1) at 0530 h. Mosquitoes that were unable to fly were subtracted from the number released.

During each of the three release nights, subsamples of the wild-caught females (pre-release populations) were dissected for parity determinations using the Polovodova technique (Detinova, 1962). These parity rates were subsequently used to compare with rates observed in recaptured populations. Christophers' and Sella's stages for each female were recorded to measure changes in ovary follicles as well as the status of blood digestion (Detinova, 1962). Uncontracted ovarian sacs were checked as an indicator of recent oviposition.

Mark-Release-Recapture Studies: Reared Females. A series of mark-release-recapture studies was conducted with laboratory-reared progeny of wild-caught engorged females. The engorged females were collected from horse-baited traps, taken to the laboratory, and held for 48 hours for egg development. The gravid females were stimulated to lay eggs by pulling off one of their wings and placing the mosquito on the surface of a bowl of water (R. C. Wilkerson, pers. comm.). The eggs were subsequently collected and placed on the water surface in 30 x 18-inch trays. The eggs hatched and larvae were fed a mixture of chicken food (®Alpezur), dry *Spirulina* algae and plant fish food. As pupation occurred, the pupae were collected and placed in a 60 x 60 x 60-cm aluminum mosquito cage. Adult emergence took place variably from early to late night.

Each morning the newly emerged males and females were sorted, counted and transferred to cardboard containers where they were marked in the same manner as the field-caught populations. In the afternoon, when mosquitoes were about 18-24 hours old, they were taken to site B (Fig. 1) and released. Individuals unable to fly were discarded. All females were uninseminated at the time of release as *An. pseudopunctipennis* from this area will not voluntarily mate under the specified laboratory conditions (pers. observation, IF). Attempts at recapture were initiated the first night following release of marked populations. Similarly, a recapture period of nine days was employed for this series of mark-release-recapture studies. Each recaptured female was dissected for determinations

of parity and insemination. In addition, 1.5 x 1 x 1.5-m pit shelters (World Health Organization, 1975) were dug next to each horse-baited trap in an attempt to recapture marked male specimens. Every morning the pits were thoroughly examined for marked males.

Length of the gonotrophic cycle for these laboratory-reared females was calculated as the number of elapsed days from the time the first blood meal was taken to the time that parous females were recaptured.

Temporal Progression of Oogenesis

Egg development in wild-caught engorged females was studied under field conditions during a 60-hour observation period. A group of 150 unmarked and freshly fed females was separated from collections made at intervals throughout the night. This population was placed in a 3.785-liter cardboard container and furnished with a cotton pad soaked with a 10% sugar water solution. The cage was then placed in a pit shelter to aproximate the conditions of a natural resting site. Midnight was taken as the starting point of this study (mid-point of the collection intervals). Subsequently, at six-hour intervals throughout the 60-hour observation period, a batch of ten females was randomly selected and dissected to record Sella's and Christophers' stages. Temperatures and relative humidity in the pit shelter was recorded at six-hour intervals. One study was conducted for each of the early and late dry season periods.

Assessment of Pre-gravid Rates.

Pre-gravid development was defined by Gilles (1955) as a state of arrested egg development following a blood meal, which occurs in females that require more than one blood meal for completion of oogenesis. There were indications from our earlier studies, as well as reports in the literature, that some specimens of *An. pseudopunctipennis* required more than one blood meal for completion of egg development (Baerg, 1971; Darsie and Lopez, 1982). To study this phenomenon, we held ten batches of wild-caught, fully-fed females in the insectary for 48 hours at 24-26 °C and relative humidities of 60-70%. Egg development was completed during this 48-hour holding period. The females were subsequently anesthetized with chloroform and examined individually. A specimen with a whitish abdomen was classified as gravid, whereas a Sella 1 female with a dark abdomen was classified as pre-gravid (Gillies, 1955). The same methods were employed with a second sample of females collected over five different nights.

In this case, the pre-gravids were dissected after the 48-hour holding period to document the parity rate of pre-gravids. The Polovodova technique was used for the parity rate determination.

A relationship between reduced body size and multiple feedings per gonotrophic cycle has been demonstrated for other anophelines (El-Akad and Humpreys, 1990). The pre-gravid phenomenon seemed a prominent component of *An. pseudopunctipennis* population dynamics. Consequently, we measured wing and body length of individual specimens from representative samples of gravids and pre-gravids to define the relationships of body size to blood meal requirements for egg production. Measurements were made by use of a dissecting microscope. Again, we held blood-engorged females under laboratory conditions for 48 hours before they were classified as gravids or pre-gravids or before they were measured. The means of the body and wing measurements of both types of females were compared by a *t*- Test for independence (SYSTAT, 1989).

Survivorship

Two analytical methods were used to estimate adult daily survival of *An. pseudopunctipennis* females in the field. For Davidson's formula (1954), we took the square root of the average parity rate ($\sqrt[\text{gc days}]{\text{parous rate}}$) if the gonotrophic cycle was completed in two days, or the third root if it required three days, *etc*. The horizontal method employs transformations of natural log plus one (x+1) of daily recapture records in regression analysis versus time. The antilog of the regression coefficient was then used as an estimate of survivorship (Gillies, 1961). An analysis of variance test for significant linear regression was calculated for each slope in each of the nine marked series. Subsequently, covariance analyses were calculated for differences between slopes for each mark-release-recapture series (Zar, 1984). Survivorship estimates for different series were pooled if there were no significant differences between slopes (Reisen, 1978).

RESULTS.

Gonotrophic cycle

Wild-Caught Females. In January 1991 (early dry season), 3,311 field-caught *An. pseudopunctipennis* females of mixed physiological age were marked and released. Of all released females, 5.7% were recaptured during the nine days of collections following release of the marked populations (Table 1). Numbers of engorged females captured per day varied from 991 to 1,342 from a total of five horse traps (mean of 1,104 females per night or 221 females per trap per night). Recapture rates for each of the three released populations were 5.1, 5.2, and 6.9 %, respectively. A few marked females were found during night 1, and two specimens contained fresh blood. The largest total number of marked females (116) were recaptured during night three post-release (Fig. 2). This pattern was consistent in all three released populations making up the early dry season series. A second smaller peak could be seen on day six in the red group (Fig. 2) and in the pooled data (Table 1).

A second series of mark-release-recapture studies was conducted in the late dry season (April). A total of 3,774 females was marked and released on three separate days. Of these, 5.4% were recaptured in the horse-baited traps (Table 1). The daily capture of unmarked specimens varied from 1,060 to 1,552 freshly fed females (mean of 1,258 females per night, or 252 females per trap per night). The recapture rate for marked specimens varied from 2.8 to 8.5%. As in the January experiment, we recaptured some engorged females during nights one and two post-release. Again, the largest numbers of marked females (115) were consistently recaptured during the third night following release (Fig. 2) for each of the released populations. Smaller peaks at days six and nine were documented only for the red group of mosquitoes (Fig. 2).

In the early dry season series of mark-release studies, we recaptured a total of seven marked females returning to a host during the first two days following their release. These females contained partially digested blood. Six of the seven females were recaptured during the first 24 hours post-release. We did not observe blood-fed females returning for a second blood meal in the late dry season series.

In the early dry season series, the first marked females with uncontracted ovarian sacs were recaptured on day three post-release. About 25.0% of all 188 recaptured females in subsequent collections had uncontracted sacs. In the late dry

season series, we observed uncontracted sacs as early as the second day post-release. An average of 21.0% of all 204 recaptured females in subsequent collections had uncontracted sacs.

During the early dry season series, in January, we noted changes in pre-release to post-release parity rates of 67% to 89% (Table 2). These rates were significantly different (X^2 =17.89, P<0.001). Rates during the late dry season series, in April, changed from 69.1 to 89.0% (X^2 =20.95, P<0.001).

Mark-Release-Recapture of laboratory-reared females. The 2,977 insectary-reared females used in these studies were about 24 hours old during the first night post-release. Most of these females, 39 (51.3%), took a blood meal during the first night post-release (Table 3). In total, only 2.6% of the released populations was recaptured during nine days of collections. A small peak in numbers recaptured was observed on day four in each of the three released populations; coincidentally, this was when marked, parous females appeared in the collections. The elapsed time between blood meals was 96 hours. After day four, the parity rates remained high in all subsequent collections for all three marked populations. The insemination rate was low in collections for days one and two; but reached a peak (60%) in day three collections. Insemination rates remained stable through all subsequent collections.

Although we marked about 2,700 males, only three were recaptured resting in pit shelters (less than 0.1%).

Temporal Progression of Oogenesis

A group of 150 naturally blood-fed, wild-caught females were held under field conditions and examined at six-hour intervals to document the temporal progression of egg development. Ten percent of these females were classified as Christophers' 5 as early as 42 hours post-engorgement (Fig. 3). During this study, the average temperature at midnight was 17.3 ^{o}C, and the average temperature at noon was 30 ^{o}C. The relative humidity was as high as 92% at night, and as low as 52% during the day.

The study was repeated in the late dry season (end of March). Oogenesis progressed more rapidly during this test with five of ten females presenting Christophers' stage 5 eggs as early as 36 hours post-engorgement (Fig. 3).

Pre-gravid Rates

Approximately 25% of 1,026 naturally blood-fed, wild-caught females underwent arrested egg development, and their abdomen appeared as Sella 1 (unfed, not gravid) within 48 hours post-engorgement (Table 4). The study populations were selected from a total of ten nights of collections and the pre-gravid rates varied in these ten samples from 6.0 to 43.0%. We observed a 21.9% pre-gravid rate in a second population of 1,195 females from another five-night series of collections. We dissected the pre-gravids from this second population and found that 84.7% were nulliparous.

Gravid females had significantly larger bodies than pre-gravid females, with mean body-lengths of 4.91 mm (n = 114) and 4.78 mm (n =167), respectively (t =2.481, df=279, P=0.014). Likewise the mean wing-length of gravid females (4.09 mm) was significantly different from pre-gravid females (3.96 mm) (t =2.730, df=279, P=0.007).

Survivorship

We used a three-day gonotrophic cycle in calculating daily survival rates with Davidson's formula (1954). The parity rates used in these calculations were derived from field-collected, unmarked specimens (Table 2). Daily survival in the early dry season was 0.8750, whereas a slightly higher estimate of 0.8840 was calculated for the late dry season trial.

Alternatively, we used data from the mark-release-recapture studies to calculate daily survival rates based on the horizontal method. The sequence of studies included in this report provided clear evidence of subpopulations of *An. pseudopunctipennis* mosquitoes, mostly nulliparous, that requires two blood meals for completion of the gonotrophic cycle. Some members of these subpopulations reappeared within 24 hours post-release for a second blood meal. Consequently, in calculating the regression lines, we deleted recapture data from day one post-release (representing only 3.7 and 1.5% of total collections in both experiments) to reduce variance and to achieve homoscedasticity in the regression analysis (Zar, 1984).

An estimated daily survival rate of 0.6900 was computed from the pooled regression coefficient (Table 6) for early dry season data. The slope was significantly different from 0 (F=6.236, df=1,6, P<0.05). When data from the three released populations were analyzed separately, only the green group showed a linear pattern. However, no significant differences (P<0.05) between slopes were

detected in covariance analyses.

The pooled survivorship estimate for the late dry season series was 0.6838. This result was not significantly different from the early dry season in a *t*-Test for paired slope comparisons (Zar, 1984). The late dry season regression coefficient was also significantly different from 0 in the ANOVA test (F=7.813, df=1,3, P<0.05). Again, no significant differences between slopes were detected with covariance analyses.

A pooled estimate of a 0.7542 daily survival rate was calculated for laboratory-reared females that were marked, released and recaptured. The regression coefficient was significantly different from 0 (F=10.646, df=1,7, P<0.05) for pooled data, and for two of the three study populations composing this series. The covariance analyses showed no significant differences (P<0.05) between slopes of the three study populations.

DISCUSSION

The horse-baited trap proved to be an excellent method for collecting large numbers of engorged females and for ensuring a high recapture rate of marked *An. pseudopunctipennis* females. In two series of capture-mark-release studies, more than five percent of the marked specimens were recaptured.

Prior to the studies included in this report, the actual length of the gonotrophic cycle of *An. pseudopunctipennis* was unknown. Although Vargas (1962) suspected that *An. pseudopunctipennis* had a gonotrophic cycle of four days, no quantitative field studies had been reported. In the mark-release-recapture series a three-day gonotrophic cycle was documented for both the early and late dry seasons. The results for all six released and recaptured populations were similar (Figure 2), despite differences in average temperatures of 2-4 °C from the early to late dry season.

Although *An. pseudopunctipennis* is considered a mountain species (Hacket, 1945), relatively little is known about the effects of ambient temperature on its biology. Davis (1928) reported accelerated post-emergence feeding by *An. pseudopunctipennis* during hotter months in northern Argentina, but he made no observations on the length of the gonotrophic cycle. Our experience during early, cooler and late, warmer, dry season indicates that this species' tolerance is variable, but low ambient temperature should be an important component of its adaptation to the foothill and mountain environments.

Studies on the temporal progression of oogenesis in *An. pseudopunctipennis* females were conducted to help determine the time when gravids might be able to oviposit. The test populations were held in pit shelters to simulate their natural resting sites. During the early dry season study, some females had produced fully mature eggs (Christophers' 5) as early as 42 hours post-engorgement (Fig. 3). This 42-hour period corresponded to the early evening hours of day two in the early dry season mark-release-recapture studies. However, the actual numbers of females recaptured during the night of day two were low (Table 1). Furthermore, uncontracted ovarian sacs in recaptured females were not detected until the night of day three post-release, which was also the night the largest numbers of marked females were recaptured. Consequently, we think the eggs of marked and released females became fully developed during the night of day two. Subsequently, the females deposited their eggs sometime during day three, and sought hosts for blood meals during the night of the same day.

Ambient temperatures were higher during the late dry season studies, so fully developed eggs were observed in specimens as early as 36 hours post-engorgement. The 36-hour interval corresponded to noon of day two. In the latter studies, a larger number of marked females was recaptured during the night of day two post-release, than in the early dry season study (Table 1). Approximately 25% of these recaptured females had uncontracted sacs (Table 2). Nevertheless, the mark-release study indicates that most, 72.0% (Day 2 + Day 3), of the released females took 36 to 54 hours to complete oogenesis and egg deposition. Consequently, the overall pattern of gonotrophic development in the late dry season populations followed the same general pattern as documented for the early dry season populations; *i.e.*, most females returned for another blood meal during the night of day three post-release.

The lowest recapture rate (2.6%) was obtained with the released populations of insectary-reared *An. pseudopunctipennis* females. The low recapture rate may reflect some loss of population vigor or behavioral modification resulting from artificial rearing conditions. It was not surprising to find that *An. pseudopunctipennis* females took their first blood meal as early as 18-24 hours post-emergence. Davis (1928) reported blood-feeding by *An. pseudopunctipennis* during late summer in northern Argentina as early as 12 hours post-emergence. The finding of a four-day gonotrophic cycle in insectary-reared populations was consistent among the three replicates. Prolongation of the gonotrophic cycle might be explained by the lack of insemination in the first days following release. Insemination is a prerequisite for ovarian development in some species; *e.g.*, *An. subpictus* (Roy, 1940). The insemination rates in females that were recaptured in the first couple of days post-release were very low. Maximum insemination rates in these insectary-reared populations were observed in gravid females recaptured at 72 hours post-release. A second factor contributing to a prolonged gonotrophic cycle was the need of a second blood meal in about 60% of all nulliparous females. The basis of the latter estimate is presented below.

Gillies (1955) described the pre-gravid rate as the proportion of females that undergo gonotrophic discordance following a blood meal. Gillies attributed gonotrophic discordance to a need for more than one blood meal per gonotrophic cycle. We documented pre-gravid rates of 22-25% for our study populations of *An. pseudopunctipennis* (Table 2). These rates were similar to the 20% pre-gravid rates reported by Gillies for *An. funestus* in Africa. Similarly, the pre-gravid populations were composed mostly (85%) of nulliparous females (Table 4). These

data were used, in combination with data on natural parity rates (Table 2) and parity rates of pre-gravids (Table 4), to estimate the per cent of nulliparous females that require a second blood meal to complete their gonotrophic cycle. First, 69% of wild-caught females were parous. Therefore, of the 1,195 wild-caught females employed in the study of pre-gravids, we can expect that 826 were parous (0.69 x 1195) and 369 were nulliparous. We know that 21.9% of the total (1,195), or 262 females, advanced to a pre-gravid stage with a single blood meal. When the pre-gravids were examined 84.7% were nulliparous, for a total of 222 females (262 x 0.847). We expected that only 369 of the total test population were nulliparous at the beginning of the study. Therefore, about 60% (222/369) of the nullipars required more than one blood meal to complete oogenesis.

El-Akad and Humprey (1990) linked the need for multiple blood meals per gonotrophic cycle of *An. pharoensis* females with poor larval nourishment. They demonstrated that larvae reared under stressed conditions produced smaller and weaker adults. The smaller adults required a second blood meal more frequently than normal-sized adults to advance egg development beyond a resting stage (the condition seen in pre-gravid females).

It may be true for most anophelines that limited food and habitat space in natural breeding sites will adversely affect larval development. It is equally possible that adults from such habitats will be less nourished and smaller than adults arising from unstressed conditions. Although the effects of breeding sites on size of adults were not investigated, we did show that pre-gravid *An. pseudopunctipennis* females were significantly smaller in body size and wing length than gravid females.

Natural populations of pre-gravids were probably represented by the recaptured females from nights one and two during both early and late dry season studies (Table 2). Interestingly, some of these females from the early dry season contained partially digested blood. Perhaps, higher ambient temperatures during the late dry season experiment increased the blood digestion rate; consequently, no bloodfed individuals were recaptured during this experiment.

We have opted for a very cautious interpretation of our estimates of daily survival probabilities. Estimates derived from the two different methods of calculating daily survivorship were very different. Daily survival probabilities of pooled marked series obtained from regression analyses were 0.6900 and 0.6838 for the early and late dry seasons, respectively. These figures were used to calculate the per cent of vector populations surviving a nine-day period for the

extrinsic incubation of *Plasmodium vivax* parasites (daily probability[9] X 100)(MacDonald, 1957). Accordingly, 3.5% and 3.2% of *An. pseudopunctipennis* females would survive up to nine days for the early and late dry seasons, respectively. Given the low population densities (Chapter 4) of these vectors in the Tapachula foothills, these survival estimates would probably be insufficient for maintaining malaria transmission in the study area. In contrast, the probabilities of surviving for nine days based on daily survival probabilities calculated with Davidson's method were 30.0%, and 32.1% for the early and late dry seasons, respectively. Such variable results in estimates of survivorship by both Davidson and the regression methods have been reported by other investigators. These variations have been attributed to variations in sampling methods and variance in natural mortality. For example, the method of regressing daily recaptures versus time was originally proposed as a method to measure daily losses (emigration plus mortality). In our study populations, the daily decline in numbers was relatively constant, and so it was mathematically independent of age ($\beta \neq 0$, $P<0.05$, slope tests for homogeneity or analyses of covariance). Consequently, we thought the regression method could provide a reasonably accurate estimate of daily survival. Charlwood and Graves (1986) compared the same method with deterministic and stochastic models and concluded that it provided an acceptable estimate of daily survival. Reisen, *et al.*, (1982) compared Davidson's method with the horizontal (cohort-specific or regression method) and vertical (time-specific or number of gonotrophic cycles) approaches. Reisen found, as in this study, that Davidson's method over-estimated and that the regression method under-estimated daily survivals if losses and emigrations were included. He concluded that for *An. culicifacies* and *An. stephensi* in Pakistan, the regression of the number of females in each gonotrophic cycle provided the most appropriate estimate for epidemiological purposes.

More recently, Clements and Paterson (1981) reported that mosquito mortality is not independent of age, and that estimates of daily survival, which include an age-dependent factor, will differ from estimates based on the classical exponential model. Clearly, there are many uncertainties associated with the different methods for estimating daily survival rates. Consequently, we are inclined to consider our estimates as range limits for some true intermediate values. We expect that the true values fall between the lowest result given by the regression method and the highest result given by Davidson's method.

The survival rate of 0.7452 for insectary-reared females was higher than

the rate for the wild-caught study populations. Additionally, the recapture rate of 2.6% was much lower than recapture rates for released populations of wild-caught females. We also experienced a higher daily loss rate for insectary-reared than for wild-caught populations. The males seemingly emigrated from the area so quickly that we recaptured only three of 2,700 marked specimens, although sampling bias may have contributed to this low recapture rate. Given that insectary-reared mosquitoes can be expected to behave differently from wild-caught females, the accuracy of our estimated survival rates for the insectary-reared populations should be interpreted with caution.

The presence of pre-gravids in the study populations introduced other sources of error, as discussed by Mutero and Birley (1989), in our estimates of survivorship. At this point we can only conclude that further refinements in methods of estimating survivorship are needed.

The physiological factors that affect requirements for length of gonotrophic cycles, such as environmental stimuli regulating female oviposition, sperm stimulation for oocyst development, *etc.*, are important components of vector biology. We need to know more about these factors to understand better the reproductive biology of *An. pseudopunctipennis* mosquitoes, and we need additional information on the role of pre-gravid mosquitoes in the dynamics of malaria transmission.

SUMMARY

Mark-recapture trials were conducted to determine the length of the gonotrophic cycle and survivorship of *An. pseudopunctipennis* mosquitoes in the Tapachula foothills of southern Mexico. Attempts with wild-caught females were conducted separately in the early and late dry seasons to check for seasonal differences. A total of 5.4 to 5.7% of all marked and released females were recaptured. A three-day gonotrophic cycle was characteristic of wild-caught females in both parts of the dry season. Similar studies with insectary-reared, nulliparous females were performed to check for effects of physiological age on the experimental results. The gonotrophic cycle for the insectary-reared females was four days. About 25% of ten separate populations of wild-caught, engorged females underwent gonotrophic discordance (known as pre-gravid females) with only one blood meal. Nearly 85.0% of the females classified as pre-gravids were nulliparous. In comparisons of body size and wing length of gravids versus pre-gravids, the pre-gravids were significantly smaller in respect to both parameters. Remarkably different estimates were obtained with the horizontal and vertical (Davidson's) methods of estimating daily survival rates. The problems of estimating survivorship are discussed.

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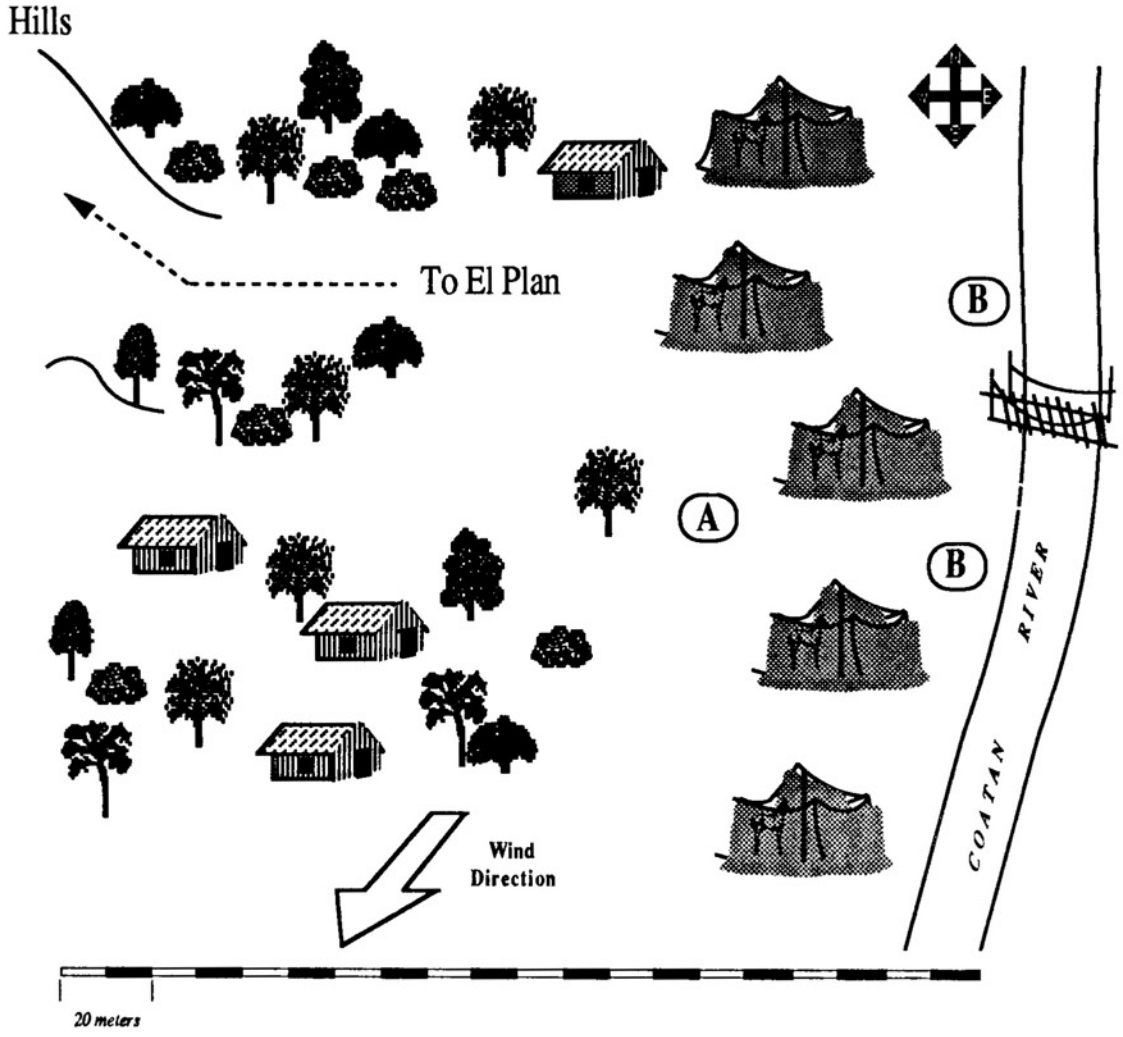

Figure 1. Map of study site along the margins of the Coatan River in the Tapachula foothills, Tapachula, Mexico. Release sites are marked by letters; A is for field-caught populations and B is for insectary-reared populations.

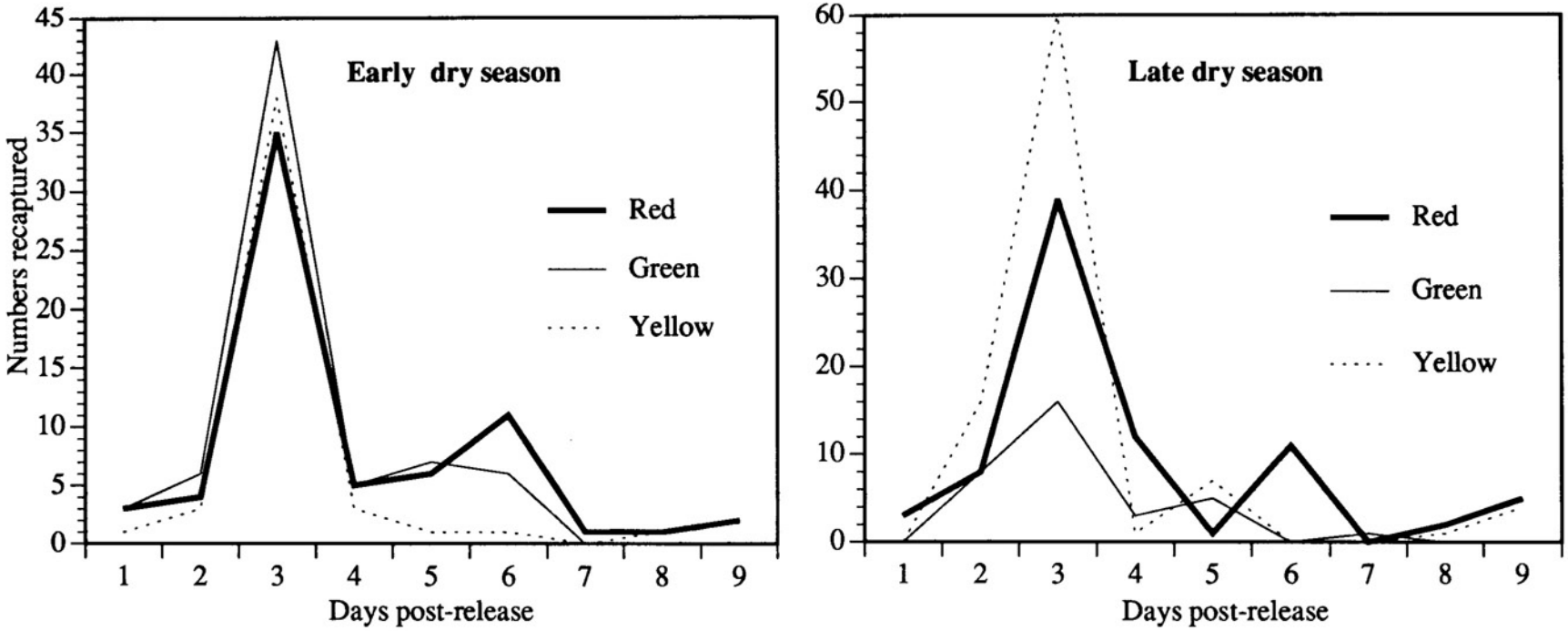

Figure 2. Numbers of marked *Anopheles pseudopunctipennis* females recaptured per day for 2 series of mark-release-recapture studies during early (January) and late (April) dry season. The three color groups, red, green and yellow, represent separate marked and released populations during a series of three nights of releases followed by 9 nights of collections to recapture marked specimens. Studies were conducted in the foothills near Tapachula, Mexico in 1991.

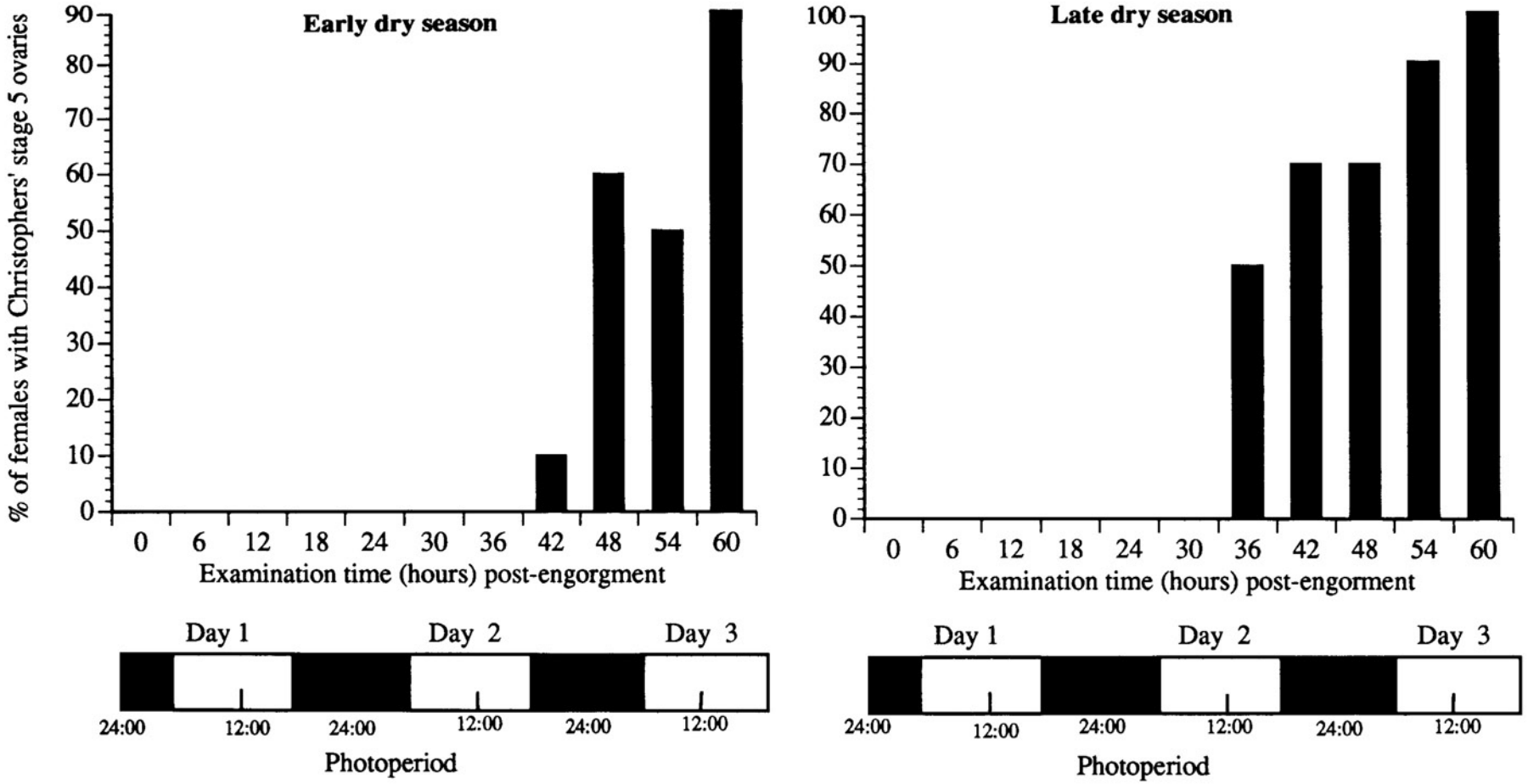

Figure 3. Percentages of samples of *Anopheles pseudopunctipennis* females with Christophers' stage 5 ovaries are plotted by time post-engorment. The lower bar illustrates the correspondence between hours post-engorment and natural photoperiod at the study site near Tapachula, Mexico. Wild-caught, naturally bloodfed females were maintained in pit shelters at the study site; and 10 specimens were examinec for Christophers' stages of egg development at each 6-hour interval. One study each was conducted in the early (January) and late (April) dry season of 1991.

Table 1. Numbers of marked *Anopheles pseudopunctipennis* females recaptured per day post-release in each of two series of mark-release-recapture studies. Data on recaptured specimens are arranged in a single sequence of days post-release. Studies were conducted in the Tapachula foothills, Tapachula, Mexico during the early (January) and late (April) dry season of 1991.

	Early dry season				**Late dry season**			
	Number recaptured				Number recaptured			
	Color groups				Color groups			
Days after release	Red (991)*	Green (1342)	Yellow (978)	Total (3311)	Red (1552)	Green (1162)	Yellow (1060)	Total (3774)
1	3	3	1	7	3	0	0	3
2	4	6	3	13	8	8	16	32
3	35	43	38	116	39	16	60	115
4	5	5	3	13	12	3	2	17
5	6	7	1	14	1	5	7	13
6	11	6	1	18	11	0	0	11
7	1	0	0	1	0	1	0	1
8	1	0	1	2	2	0	1	3
9	2	0	2	4	5	0	4	9
Recapture rates	68 (6.9%)	70 (5.2%))	50 (5.1%)	188 (5.7%)	81 (5.2%)	33 (2.8%)	90 (8.5%)	204 (5.4%)

*Number of females marked and released.

Table 2. Parity rates of *Anopheles pseudopunctipennis* females used in two series of mark-release-recapture studies. Parity rates are reported for populations immediately before they were marked and released, and for recaptured females from each of nine days post-release. Studies were conducted in the foothills near Tapachula, Mexico during the early (January) and late (April) dry seasons of 1991.

	Early dry season					Late dry season				
				Parity rates					Parity rates	
Days after release	Numbers recaptured	% Blood-fed	% With sacs*	Before release	After release	Numbers recaptured	% Blood-fed	% w/Sac*	Before release	After release
1	7	86.0	0.0	65.5% (19/29)	100	3	0.0	0.0	86.0% (43/50)	67.0
2	13	15.4	0.0	74.3% (29/39)	61.5	32	0.0	25.0	72.0% (36/50)	72.0
3	116	0.0	21.5	61.2% (19/31)	90.0	115	0.0	23.0	49.0% (24/49)	96.0
4	13	0.0	23.1		100	17	0.0	29.0		71.0
5	14	0.0	36.0		93.0	13	0.0	8.0		93.0
6	18	0.0	61.1		94.0	11	0.0	0.0		82.0
7	1	0.0	100		100	1	0.0	0.0		100
8	2	0.0	50.0		100	3	0.0	0.0		100
9	4	0.0	0.0		50.0	9	0.0	22.0		100
	188	4.3	25.0	67.0% (67/99)	89.0	204	0.0	21.0	69.1% (103/149)	89.0

*Uncontracted ovariole sac

Table 3. Numbers recaptured, parity and insemination rates for marked and released insectary-reared *Anopheles pseudopunctipennis* females*. Numbers recaptured are reported for three populations of marked specimens following their release in the foothills area near Tapachula, Mexico in April, 1991.

Days after release	Numbers recaptured				Per cent parous	Per cent inseminated
	Color groups					
	Yellow (921)**	Red (915)	Green (1141)	Total (2977)		
0†	20	13	6	39	0.0	5.1
1	5	1	8	14	0.0	14.3
2	2	2	1	5	0.0	60.0
3	2	0	0	2	0.0	100
4	3	1	3	7	100	85.7
5	1	0	1	2	100	100
6	0	0	2	2	100	100
7	1	0	2	3	100	100
8	0	0	2	2	100	100
Recapture rate	34 (3.7 %)	17 (1.8%)	25 (2.2%)	76 (2.6%)		

*All specimens were freshlly emerged at the time they were marked and released.
**Number of females marked and released
†Release night

Table 4. Pre-gravid rates of engorged *Anopheles pseudopunctipennis* females collected in the foothills near Tapachula, Mexico during February and March 1991. Rates were obtained by holding engorged females for 48 hours, and then by examining and classifying specimens as "gravid" or "pre-gravid" based on appearance of the abdomen. Pre-gravids in study 2 were dissected for parity determinations.

Study population No. 1			Study population No.2				
Collection date	Engorged females	% Pre-gravid	Dissection date	Engorged females	Pre-gravid females (%)	Numbers nulliparous	Numbers parous
11-Feb-91	326	33.1	11-Mar-91	329	54 (16.4)	50	4
12-Feb-91	73	38.4	12-Mar-91	196	43 (22.0)	37	6
13-Feb-91	73	43.0	13-Mar-91	173	39 (22.5)	35	4
18-Feb-91	109	32.1	14-Mar-91	270	61 (22.6)	50	11
19-Feb-91	150	18.0	15-Mar-91	227	65 (28.6)	50	15
20-Feb-91	95	26.3					
21-Feb-91	143	13.0					
22-Feb-91	184	6.0					
23-Feb-91	177	25.0					
25-Feb-91	36	36.1					
Totals:	1026	25.0		1195	262	222	40
Rates:					(21.9%)	(84.7%)	(15.3%)

Table 5. Numbers measured, means and 95% confidence intervals for body and wing lengths of gravid and pre-gravid *Anopheles pseudopunctipennis* females. Studies conducted with females caught in the foothills near Tapachula, Mexico, in March 1991.

	Gravid		Pre-gravid		
	mean	95 % C.I.	mean	95 % C.I.	
Body length (mm)	4.91	4.87-4.95	4.78	4.75-4.80	P< 0.016 *
Wing length (mm)	4.09	4.05-4.12	3.96	3.93-3.98	P< 0.007 *
n	114		167		

* Significantly different, independent *t*-Test

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CHAPTER 3

Host selection patterns of *Anopheles pseudopunctipennis* under regular insecticide spraying situations in southern Mexico

INTRODUCTION.

Information on the feeding patterns of arthropod vectors is important to medical entomologists and epidemiologists for understanding host-vector relationships and the dynamics of disease transmission. Host selection patterns of malaria vectors have been quantified by the relative frequency of blood from different host-types in samples of engorged mosquitoes by place (a locality or biotope) and time (Boreham and Garret-Jones, 1973). Within this framework, the human blood index (HBI) is used to estimate human-vector relationships. The HBI is defined as the proportion of freshly engorged anophelines found to contain human blood (World Health Organization, 1963; Garret-Jones, 1964; Garret-Jones and Shidrawi, 1969). This index is useful for epidemiological assessments of malaria control program effectiveness (Garret-Jones, 1964), as well as a component of vector capacity (Garret-Jones and Shidrawi, 1969).

Many different serological methods are available for identifying bloo meal hosts; *e.g.*, ring precipitine, agar gel diffusion, fluorescent antibody technique, passive hemagglutination inhibition technique, and the enzyme-linked immunosorbent assay (ELISA). Each of these methods is characterized by strengths and weaknesses relating to assay sensitivity versus specificity (Washino and Tempelis, 1983). Although it is important to select the "right" assay, an additional concern should be the methodology of where, when and how samples are collected from the field. Biased data and erroneous conclusions can be expected when such factors as time and location of sampling, and density, distribution and availability of hosts are not carefully considered in the study plan. Vectors opportunistically switching host selection patterns from animals to humans in response to changes in host density and availability are of special interest in malaria transmission studies (Garret-Jones,*et al.*, 1980). An understanding of the normal variations in a vector's host preferences will facilitate efforts to detect any significant switch in host selection patterns. Knowledge of normal variations in host preferences are particularly useful in studies to determine the effects of insecticides on human-vector contact. The excito-repellent effect of DDT on house-entering vector populations is widely recognized by field workers in the malaria endemic countries (Garret-Jones, 1964). This repellent effect is particularly important because it results in reduced numbers of vectors resting indoors and changed feeding patterns.

Included in this report are results of studies on the host selection patterns

of *An. pseudopunctipennis*, a primary vector of malaria in Mexico (Rodriguez and Loyola, 1989). The studies were conducted in four villages in the Tapachula foothills of southern Mexico during 1990 and 1991. Since the villages are routinely sprayed with insecticides by the National Malaria Control Program, data also are included from 1991 on the effect of house spraying on host selection patterns.

MATERIALS AND METHODS

Description of study sites

The study area is located in the foothills of El Soconusco, which is the western Pacific slope of the Sierra Madre mountain range. This area is 30 km from the city of Tapachula in Chiapas, Mexico. Chiapas is the southernmost state in Mexico and borders Guatemala along the Suchiate River. The climate of the study area is tropical with distinct wet and dry seasons, May-November and December-April, respectively.

The foothills comprise an area of rugged topography where coffee is grown intensively, serving as the primary agricultural activity. The many small villages scattered throughout the foothills provide the labor pool for manually cultivating and harvesting the coffee crops. Although dogs, cats, pigs and chickens are present in the villages, there are no pastures, and cows and horses are scarce.

Four villages, at elevations of 400 to 650 m, were selected as sites for studies included in this report. Average distance separating each of the four villages is 3.5 km. All four villages are located in similar ecological settings along the Coatan River, and the river is the most important dry season breeding site of *An. pseudopunctipennis* mosquitoes. Two villages, El Retiro and El Plan, were chosen for their high mosquito densities, and two, La Ceiba and La Concordia, for low mosquito densities (Loyola, 1988, unpublished data). A census of animal populations was conducted within the four villages.

Insecticide treatments

Personnel of the National Malaria Control Program in Chiapas State routinely apply DDT or Bendiocarb to walls of houses within the malaria endemic villages. In the foothills, DDT is sprayed at six-month intervals in villages where more than five malaria cases are detected by passive surveillance. Bendiocarb is used if the outbreak seems to pose a hazard for surrounding villages (Loyola, 1989, personal communication). During two years of studies, Bendiocarb [(0.4 g active ingredient (AI)/m2)] was sprayed in the four study villages in September 1989, and again in May 1990. DDT (2g AI /m2) was sprayed in houses during late January and early February 1991.

Field collections.

During the first year, resting collections were conducted from mid-January to May 1990. Each village was sampled during one week each month. For two hours every other morning a team of four people searched a minimum of ten houses. A different ten houses per village were sampled during each day of collecting. A thorough examination was made of walls, cracks and furniture using flashlights and mouth aspirators to spot and capture resting mosquitoes. On alternate days, shelters surrounding the human dwellings, such as chicken houses, coffee plants, orchards, ground depressions, rock piles, latrines, *etc.* were searched for resting adult anophelines. Captured specimens were held in pint cardboard containers and taken to the laboratory where they were sorted by sex and classified as fed, unfed or gravid. The abdomens of freshly fed females were smeared onto Whatman No. 2 filter paper. The papers were then dried, wrapped with glassine paper and stored at 4 °C until processed for blood meal identification at the end of the study.

The same procedures of indoor and outdoor collections of resting mosquitoes were continued during the dry season of 1991 (started from December 1990 to May 1991). Additionally, in an attempt to increase the sample size of bloodfed mosquitoes, ten 1.5 x 1.2 x 1.0-m pit-shelters were dug in each of the four villages; they were located in the backyards of some houses and in the center of some neighboring coffee plantations. A separate team of collectors (not the same team that conducted collections from indoors and natural shelters) searched the pits for resting anophelines each morning during weekdays. This sampling effort was conducted from late December 1990 to May 1991.

Blood meal identification.

An indirect ELISA for blood identification (Loyola, *et al.*, 1990) was used to screen the blood meals smeared on filter papers. The samples were eluted overnight at 4 °C with 200 ml of a phosphate buffer saline solution (pH 7.2 PBS). Five ml of each eluted sample were placed with 50 ml of coating buffer (sodium bicarbonate 35 mM, pH 9.6) in six wells of a polystyrene microtiter plate (Dynatech Laboratories, Inc., Alexandria, Virginia) and incubated for 1 hour at room temperature. After blocking unreacted sites with 2.5% dry milk in 7.2 pH PBS, the wells were screened against a bank of antibodies (Sigma Chemical Co., St. Louis, Missouri) for identifying blood from human, horse, dog, pig or chicken, and this

was followed by horseradish peroxidase-conjugated goat serum anti-rabbit IgG. Color was developed using 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Sigma Co.) as a substrate. Blood samples of the host species were dried on filter paper and used during tests as positive controls. A test was positive when its absorbance value was higher than two times the mean of five negative controls (consisting of male and unfed female *An. pseudopunctipennis* mosquitoes). Mixed blood meals represented two or more different hosts.

Data analysis.

Two measurements of *An. pseudopunctipennis* blood-feeding habits were calculated from blood meal identification data. The human blood indices (HBIs) were calculated from freshly engorged specimens collected resting inside of houses and from outdoor sites. Specimens from out-of-doors consisted of individuals from natural resting sites and collections from the artificial pit shelters. The HBI was calculated as the unweighted mean of the proportion of specimens with human blood that were collected from indoor and outdoor locations. This index is considered an unbiased estimate because it uses percentages instead of raw number; it was also computed to account for bias introduced by disparities in the spatial distribution of resting females. The weighted HBI or crude mean is the sum of indoor and outdoor numbers containing a specific host blood meal divided by the total numbers of indoor-outdoor bloodfed mosquitoes (Garret-Jones, 1964).

The forage ratio (FR) was the second measure, which quantifies vector preference for a particular vertebrate host rather than other available hosts (Boreham and Garret-Jones, 1973). The FR was calculated by determining the percent of *An. pseudopunctipennis* females containing blood of a particular host, divided by the percent of the total available host population represented by that particular host (Hess, *et al.*, 1968). A FR of one, or near one, indicates neither preference nor avoidance of a particular host animal; FRs significantly greater than one indicate selective preference, and values less than one indicate avoidance of a host in favor of other available hosts.

As stressed by Loyola, *et al.*(1990), the HBI is an indicator of effective human/vector contact and is of epidemiological importance. The FR is more precisely an indicator of host preferences and is of biological importance.

Homogeneity chi-square tests were computed to analyze sample relative frequency comparisons. Regression and ANOVA tests were made to demonstrate

linear associations (SYSTAT, 1989).

RESULTS

Resting population densities

Populations of *An. pseudopunctipennis* are most abundant in the Tapachula foothills from mid-December to May (Chapter 3). Within this period of peak abundance, the monthly densities for indoor resting mosquitoes during 1990 were different from the 1991 season (Fig. 1). Sampling was initiated in January of 1990 and resting females were found on house walls from January to March. In contrast, in 1991 most resting females were found on house walls only in January.

A total of 1859 *An. pseudopunctipennis* mosquitoes were obtained from the two years of resting collections in the four study villages, and 45.0% of the total was engorged. In the first year, only 366 (19.7%) specimens were obtained from resting collections, whereas 1493 (80.3%) were captured at the second year. Approximately 34.0% of the captured specimens that did not contain blood were classified as unfed, 21.4% as gravid, and 54.0% as males (Table 1). The specimens tested for blood meal identification comprised 94% of all engorged females. In total, the indoor collections accounted for 12%, natural shelters accounted for 18%, and the artificial pit shelters (from 1991) accounted for 70% of the 1859 specimens obtained from resting collections. Most engorged specimens were obtained during the second year from pit shelters.

As expected, the villages with overall higher densities of *An. pseudopunctipennis* in landing collections also yielded higher numbers in resting collections. In terms of numbers tested for blood meal identification, 30% were from El Plan, and 62% were from El Retiro, whereas only 7% were from La Concordia and less than 1% were from La Ceiba (Table 1). There were no significant differences in overall numbers of females from indoor and natural shelter collections between 1990 and 1991 (X^2, $P>0.05$) this statistic excludes data from pit-shelter collections.

Human Blood Index

The proportions of females containing human blood were relatively similar by site (indoors, natural shelters outside, or pit shelters), year and village. The proportions did not seem to vary with sample size. Additionally, for a given type of collecting site, the per cent of females containing human blood was similar to the per cent with human blood in pooled samples from all four villages, for both years

(Table 2). In other words, despite differences in numbers collected, the samples were representative of overall feeding patterns.

On average, 53.7% of all specimens collected indoors in all villages during 1990 had fed on human blood, compared to 86.2% that had fed on humans in the 1991 season (Table 2). The differences between the two years were significantly different (X^2=24.3, P<0.01). The rates of human blood meals in specimens from natural shelters varied from 18.8% to 23.5% for the first and second years, respectively. These differences in annual rates were not statistically different (X^2=*0.741*, P>0.05). The annual per cent of specimens collected from pit shelters with human blood meals (23.0%) was similar to the annual rates for specimens from natural resting places (23.5%) despite great differences in relative sample size (Table 2).

Bloodmeal data from all localities was pooled to obtain a representative estimate of the overall HBI. Data were pooled in order to have at least 50 blood smears per locality, which is considered the minimum sample size representative of a local population (Garret-Jones, 1964). The pooled data were used to calculate weighted and unweighted mean HBI values. Weighted HBI estimates for the first and second years were 34.0% and 29.5%, respectively (Table 3). These HBI values for two years were not significantly different (X^2=0.368 P>0.05). The unweighted HBI values were 36.2% for the first year and 44.2% for the second; again, the differences were not significant (X^2=*0.365*, P>0.05).

Of all other available hosts, dogs seemed to be the most important blood source out-of-doors in all villages (Table 3). During two years of collections, dogs were the blood source for 29.6% to 51.3% of all engorged *An. pseudopunctipennis* females collected outside from pit-shelters and other natural resting sites. In contrast, dogs were the blood source for only 6.2% to 11.9% of the engorged females collected indoors.

Forage Ratios

Host availability was determined by a detailed census of vertebrate hosts, including humans, for each of the four study villages. The patterns of host densities within the four villages were similar.

Chickens were found to constitute more than 50% of all vertebrate hosts present within the village environments. Regardless, chickens accounted for less than 2.4% of all the identified blood meals (Table 4). Since chickens were clearly

not a factor in the overall issue of *An. pseudopunctipennis* host preferences, statistics on chicken populations are not included in the following calculations of forage ratios.

Humans were three times more abundant (76.1%) in villages than domestic animals (excluding chickens). However, the FR for humans was less than one for both years, with or without numbers of chickens, which indicates that *An. pseudopunctipennis* females fed preferentially on other available hosts. Forage ratios greater than one were calculated for all other available hosts; *viz.*, dogs, pigs and horses. The FRs for horses for the two years were 15.6 and 20.9 (Table 4). While horses represented less than 1% of all available hosts, *An. pseudopunctipennis* populations preferentially fed on this animal. The second most preferred host was the pig, with FRs of 5.8 and 7.2 for the first and second years, respectively. Dog FRs showed a weaker preference (1.6 and 1.4) than for larger domestic animals.

Spraying effect on host selection

During the second year of the study, DDT was sprayed on village house walls in late January. After spraying, densities of indoor-resting *An. pseudopunctipennis* females dropped precipitously and, to a lesser extent, densities dropped in outdoor resting sites as well (Fig. 1). Under these circumstances, pit-shelters became an increasingly valuable source of blood engorged specimens. Consequently, the collections from the pit-shelters were used to determine the impact of DDT residues on host selection patterns of the outdoor resting populations. Monthly proportions of human and animal blood meals were arcsine-transformed (Steel and Torrie, 1980) and analyzed by single linear regression (SYSTAT, 1989). A shift in host selection occurred after the villages were sprayed with DDT. In collections of engorged specimens from pit-shelters, the proportions of human bloodfed females were high before treatment (33.7%), dropped after the houses were sprayed (15.2%), and seemed to begin to increase 2-3 months later (16.1%) (Fig. 2). The animal blood proportions were, in essence, the inverse of the monthly human fed proportions, so the animal blood per cents began to increase immediately after the houses were sprayed and started to decrease 2-3 months later (Fig. 2). A negative linear relationship showing statistical significance (r=0.997, ß=-0.563, P=0.0, n=5) was found between the two variables (Fig. 2).

DISCUSSION

Resting populations

The study of host selection patterns of malaria vectors is a complex task in areas where insecticides are continuously applied to house walls for malaria control. Dramatic changes in feeding patterns and in densities of resting mosquitoes are always expected under such "real world" conditions. Additionally, bloodfed anophelines tend to seek more protected resting places where they will not be irritated or affected by the insecticide. This insecticide-induced population movement will be reflected in the results of the sampling program (Garret-Jones, 1964).

The patterns of monthly densities in the indoor resting populations of *An. pseudopunctipennis* in study villages during 1990 and 1991 were different (Fig. 1). The differences might be explained, in part, by differences in timing of malaria control measures, as well as differences in insecticides employed. During the first year the malaria control team sprayed house walls with Bendiocarb in September 1989, in response to an early-season occurrence of malaria cases (probably relapsing cases; Loyola, 1990, personal communication). This was considered "early-season", since it occurred during the wet season, and densities of *An. pseudopunctipennis* populations were at their lowest level (*An. pseudopunctipennis* population densities begin to increase during mid-December in these locations; see Chapter 3). Bendiocarb was again sprayed on the house walls in April of 1990, (Pozos, 1991, personal communication). Probably the three-months residual action of Bendiocarb (Pant, 1988) on house walls degraded from September to mid-December 1989, and this loss of insecticidal effect permitted population densities of *An. pseudopunctipennis* to increase during December 1989 and January 1990. Consequently, population densities remained high throughout most of the 1990 dry season, or until April. In the following year (1991), DDT was sprayed on village house walls during late January and early February. This application of DDT was timed to correspond with the appearance of peak vector densities. Based on data from collections of resting adults, the insecticide residues essentially eliminated the indoor host-seeking and indoor resting populations of *An. pseudopunctipennis* mosquitoes from late January until late April 1991. The collections were conducted before and after houses were sprayed with DDT.

Overall, the intensive collections of indoor and outdoor resting adults of *An. pseudopunctipennis* were relatively unproductive during the two years. For

example, all such collections during 1990 and 1991 produced only 18.8% and 14.8% of the total number of engorged females processed for blood meal identification (Table 2). However, in the second year, the pit-shelters produced about four times as many resting, engorged *An. pseudopunctipennis* females as were obtained in samples from more natural resting sites. This fruitful result indicates that pit-shelters should be used in future for further studies on the resting behavior of *An. pseudopunctipennis*.

The Coatan River is the major breeding site of *An. pseudopunctipennis* mosquitoes during the dry season. Not surprisingly, the two villages located closest to the river, El Plan and El Retiro, also provided most of the engorged females (92%) in the resting collections.

Human Blood Index

In samples from the four study villages, both the weighted and unweighted HBIs for *An. pseudopunctipennis* characterized a relatively anthropophilic vector. The unbiased estimator, or unweighted mean, was always higher than the weighted HBI during the two years (36.2 and 44.2% versus 34.0 to 29.5%) (Table 3). The reason for this relationship between weighted and unweighted HBIs probably relates to the greater effect of large percentages of human bloodfed specimens in indoors collections on the unweighted HBI. For example, in the first year, 53.7% of indoor resting females contained human blood, while 86.2% had human blood meals during the second year (Table 2). Similarly, high proportions of indoor resting *An. pseudopunctipennis* females with human blood have been found in other countries; *viz.*, Vargas (1938) found 67.6% in Temixco, Central Mexico; Davis and Shannon (1928) reported 50.0% with human blood in northern Argentina; and in Peru, Acosta (1960) found 80.6% with human blood. These data reveal a high degree of anthropophagy in indoor resting populations.

The residual effects of insecticide sprayed on village house walls probably account for some differences in unweighted HBIs between 1990 (36.2%) and 1991 (44.2%). However, the influence of insecticide treatment was more apparent in comparisons of percentages of indoor resting females containing human blood. In 1990, approximately 86.1% of indoor resting females were positive for human blood, with only 53.8% in 1991. The study houses were sprayed with Bendiocarb in September 1989. Considering that residual effect of Bendiocarb is estimated to be three months (Pant, 1988), the overall effect of the insecticide was expected to

be low during the period of peak vector density in 1990 (January through March). Clearly, the negligible effects of the insecticide residues accounted for the high percentage of females containing human blood during the dry season. Alternatively, houses were sprayed with Bendiocarb in April, 1990, and were not treated again until late January-early February 1991. Consequently, the vectors were entering houses and feeding on humans during December 1990 and early January 1991. However, after houses were sprayed with DDT in late January 1991, the indoor resting collections were negative. As a result, the percentage of human blood feeding before and after DDT treatment was 86.6% (52/60) and 0%, respectively. The unweighted HBIs could not be used for these comparisons simply because no mosquitoes were caught resting indoors. Nevertheless, we can expect that the effects of Bendiocarb and DDT will be different even in recently sprayed houses. Support for this supposition is provided by the report of Loyola, *et al.* (1990), in which they found a higher unweighted HBI (12.7 to 26.9%) in the Bendiocarb-sprayed villages versus an unweighted HBI of 3.3 to 6.8% in DDT-sprayed villages.

In these villages, coffee is the primary cash crop in the foothill region, and the low numbers of large domestic animals as alternate hosts is related to the practice of manually harvesting the coffee beans. Consequently, humans accounted for 76.1% of all available vertebrate hosts within the villages, and this seems to be the primary reason for the high HBIs (Table 4). Edman (1971) found that the feeding pattern of mosquito species within five genera corresponded to abundance of the primary hosts. Clearly, these concerns related to host density and availability emphazise the need to carefully design and interpret host selection studies. Washino and Tempelis (1983) have reviewed the biases that are commonly associated with studies of human blood indexes and determinations of host preferences. Washino and Tempelis divided the problems associated with sampling and assessment into two types: bias induced by the nature of the vertebrate host present in a specific place and bias resulting from the nature of the mosquito population being sampled. However, despite these controversial issues and difficulties, we think that under epidemic situations, blood meal identification data and HBIs should be useful for determining the level of man-vector contact and for epidemiological assessments of control programs in malaria endemic areas.

Dogs were second only to humans as a source of blood meals for *An. pseudopunctipennis* populations (Table 2). Furthermore, dogs were the second

most common host (chickens excluded) within the village environment (Table 4). Humans and dogs accounted for more than 96.0% of the *An. pseudopunctipennis* blood meals. This skewed pattern in host selection reveals a strong association of *An. pseudopunctipennis* with the domestic environment. Such an association clearly defines the human domicile as a high risk biotope for the transmission of malaria in southern Mexico.

Mixed blood meals, mainly of man-dog, dog-horse, pig-dog and man-pig, were found in less than 2.1% of all engorged specimens (Table 3). Mixed and cryptic (two or more blood meals from the same type of host in a single mosquito) blood meals may be of epidemiological importance because of the increased risk of malaria transmission posed by a vector that takes multiple partial meals, as in the case of *An. sacharovi* (Boreham and Garret-Jones, 1973). Unfortunately, we could only identify mixed blood meals if the mosquito fed sequentially on different types of hosts. In other words, we could not detect instances of a single mosquito feeding on different individuals of the same type of host. Regardless, our findings that approximately 25% of the *An. pseudopunctipennis* entered a pre-gravid stage and therefore took more than one blood meal during a gonotrophic cycle (Chapter 2) emphasizes the need to study the phenomenon of mixed or cryptic blood meals in order to understand its implications for malaria transmission.

Forage Ratios

The HBIs were indicative of very high man-vector contact under the specific conditions of host availability at the study sites. In contrast, the FRs, which are measures of biological preferences, clearly defined *An. pseudopunctipennis* as a zoophilic species (Table 4). The FRs for humans were always less than 1 (with or without chicken populations); therefore, it seems that these anophelines tend to avoid the human host. A strong host preference was consistently demonstrated for the larger mammals, such as horses (FR>15) and pigs (FR>5.8). Even when these hosts were less abundant than humans in the study villages, the large animal hosts presented a much greater surface area to host-seeking mosquitoes. This large surface area might contribute to the great differences between horse and humans FRs. Unfortunately, differences in quantity of surface areas is not included in the FR calculations. Some kind of adjustment for host surface area might better define the feeding relationships between the mosquito and its naturally preferred hosts. Larger hosts, such as horses and pigs, are known to attract most bites of *An. pseudopunctipennis* in a variety of ecological settings in Mexico; *e.g.*, Sinaloa

(Loyola, *et al.*, 1990), and Temixco (Vargas, 1938). A similar preference by *An. pseudopunctipennis* for large mammals was demonstrated in Peru by Sasse and Hackett (1950). Sasse and Hackett used a stable trap and alternated exposures of hosts to blood-seeking populations; hosts included man, goat, pig, calf and donkey. The result was that most blood meals (62.3%) were from donkey. In another comparison of horses versus cows and humans in experimental huts, horses received more than 70.0% of the bites (Vargas, 1938). The FRs for *An. pseudopunctipennis* provide additional evidence of a intrinsic preference for feeding on large mammals. Apparently, feeding on humans is partly opportunistic and reflects host availability more than host preference.

Effects of insecticides on host selection

The pre- and post-spraying changes in HBIs as a result of DDT irritability have been documented for some malaria vectors (Garret-Jones, 1964). In our case, no *An. pseudopunctipennis* populations were found resting indoors for almost three months after the houses were sprayed with DDT; therefore, comparisons of pre- and post-spraying HBIs were not possible. Regardless, the per cent of specimens containing human blood in the pit-shelter collection declined after the houses were sprayed with DDT. Similarly, the per cent containing animal blood increased. The shift in host selection patterns probably occurred because the populations were forced to feed on alternative hosts not protected by insecticide residues. The population-based reduction in human blood feeding as a result of DDT residues on house walls, as seen in Fig. 2, possibly indicates that the insecticide produces an "area effect." In other words, presence of insecticide reduces the numbers of bites on humans outside, as well as in indoor locations. Similar changes in the HBIs of populations collected from outdoor shelters have been reported for important vectors species in Africa, such as *An. funestus* and *An. gambiae* (Garret-Jones, 1964).

Insecticide repellency (detection and evasion with no contact) and irritability (reaction produced with insecticide contact) occurs in mosquito populations (Georghiou, 1972). In fact, a combination of repellency and irritability may be involved in the indoor resting behavior and the selection of blood meal hosts by *An. pseudopunctipennis* in DDT-sprayed villages. Additional studies on the behavioral responses of *An. pseudopunctipennis* to insecticide residues are needed. Such studies might help explain the persistence of malaria in this part of southern

Mexico.

SUMMARY

Studies of host selection patterns of *Anopheles pseudopunctipennis* were conducted in villages in foothills near Tapachula, Mexico. Based on two years of collections, 53.7 and 86.2% of all engorged females resting inside of houses were found to contain human blood. Estimates of weighted and unweighted Human Blood Indexes (HBIs), including data from outdoor resting collections, varied from 30 to 44.2%. Humans and dogs were the more common blood source for all *An. pseudopunctipennis* mosquitoes, accounting for 96% of blood meals tested. Results of analyzes of host preference through estimates of forrage ratios (FR) indicated that the large numbers of blood meals from humans and dogs are more reflective of host availability than host preference. A FR of less than 1 indicated that *An. pseudopunctipennis* females avoided, to a marginal extent, the human host. In contrast, FRs of 15-20 and 5-7 were indicative of strong host preference for horses and pigs, respectively. The proportion of outdoor-resting, blood-engorged females containing human blood declined markedly after houses were sprayed with DDT. This response to house spraying is attributed to an excito-repellency effect of DDT. These studies clearly define a strong association of *An. pseudopunctipennis* with domestic environment. The human-vector association is a key factor in the role of this anopheline as a major vector of human malaria.

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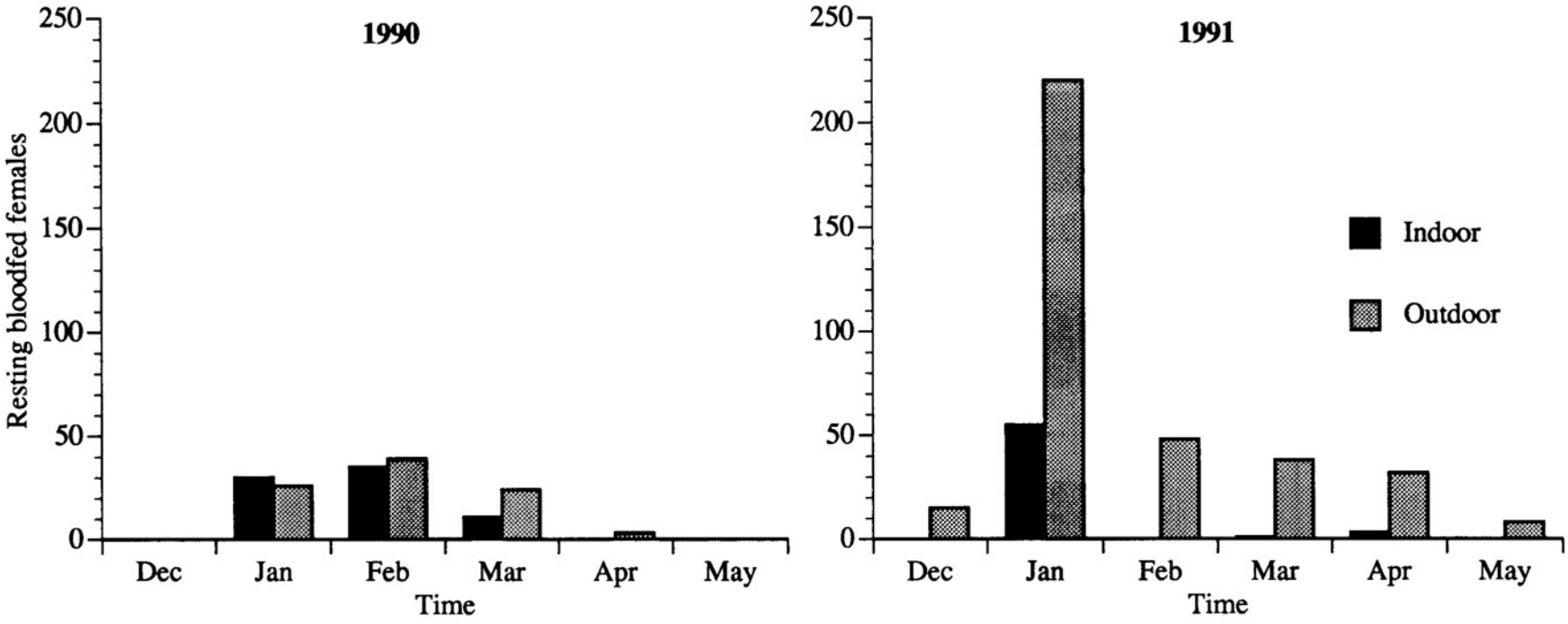

Figure 1. Monthly distribution of resting, bloodfed *An. pseudopunctipennis* females in four villages in the foothills near Tapachula, Mexico. Data were pooled for all indoor and outdoor collections of resting adults that were conducted during the dry season of 1990 and 1991. Numbers captured outdoors in 1991 include collections from artificial pit-shelters.

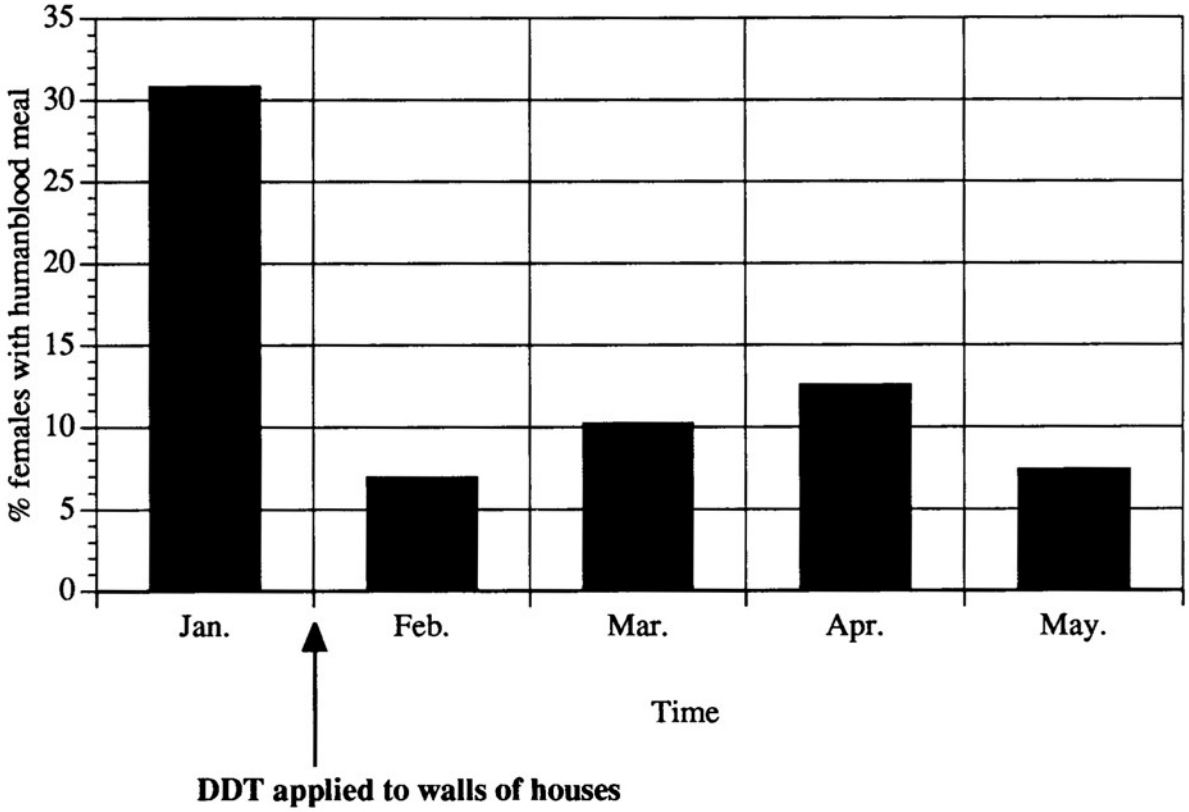

Figure 2. Percentages of *An. pseudopunctipennis* females that contained human blood before and after houses in the four villages that were sprayed with DDT. Data were compiled from routine resting collections from pit-shelters during the dry season (January to May 1991) in foothills near Tapachula, Mexico.

Table 1. *Anopheles pseudopunctipennis* mosquitoes captured in indoor and outdoor resting collections in four villages in the foothills near Tapachula, Mexico, during the dry season of 1990 and 1991. Data are presented on numbers captured, tested for blood identifications (host-type), fed, unfed, gravid and males.

	Indoor resting						Outdoor resting natural shelters					
Village			Numbers						Numbers			
1990	Caught	% Tested	Fed	Unfed	Gravid	Males	Caught	% Tested	Fed	Unfed	Gravid	Males
El Plan	56	53.6	30	23	3	5	124	8.9	45	43	36	3
El Retiro	70	51.4	43	20	7	0	101	60.4	43	57	1	11
La Concordia	1	100	1	0	0	0	4	0	2	1	1	0
La Ceiba	2	0.0	2	0	0	0	8	100	6	0	2	0
Totals	129	52.0	76	43	10	5	237	33.8	96	101	40	14
1991												
El Plan	78	75.6	51	14	13	1	8	12.5	4	2	2	4
El Retiro	10	50.0	7	3	0	1	92	50.0	53	38	1	1
La Concordia	1	100	1	0	0	0	1	100	1	0	0	0
La Ceiba	2	0.0	1	1	1	0	3	100	3	0	1	5
Totals	91	71.4	60	17	14	2	104	49.0	61	40	4	10
									(Artificial pit-shelters)			
El Plan							412	33.3	145	142	125	479
El Retiro							757	44.7	343	253	161	418
La Concordia							124	35.6	49	33	42	66
La Ceiba							5	40	2	1	2	11
Totals							1298	40.1	539	429	330	974

Table 2. Blood meal identifications from *Anopheles pseudopunctipennis* mosquitoes captured in indoor and outdoor resting collections in four villages in the foothills near Tapachula, Mexico during the dry seasons of 1990 and 1991. Data are presented on numbers that had fed on humans (Hu), horses (Hr), dogs (Dg), chickens (Chk), mixed hosts (Mx), not identified (not ID), total tested and the per cent fed on humans.

	Indoor resting									Outdoor resting natural shelters								
	Numbers by host-type									Numbers by host-type								
Villages	Hu	Hr	Dg	Pg	Chk	Mx	Not ID	Total tested	†HBP	Hu	Hr	Dg	Pg	Chk	Mx	Not ID	Total tested	HBP
1990																		
El Plan	17	6	4	2	1	0	0	30	56.7	2	3	5	0	1	0	0	11	18.2
El Retiro	19	7	3	7	0	0	0	36	52.8	12	1	35	10	1	0	2	61	19.2
La Concordia	0	0	1	0	0	0	0	1	0.0	1	3	1	3	0	0	0	8	12.5
La Ceiba	0	0	0	0	0	0	0	0	0.0	0	0	0	0	0	0	0	0	0.0
Totals	36	13	8	9	1	0	0	67	53.7	15	7	41	13	2	0	2	80	18.8
1991																		
El Plan	52	1	3	0	2	1	0	59	88.1	0	0	1	0	0	0	0	1	0.0
El Retiro	4	1	0	0	0	0	0	5	80.0	11	10	17	6	1	1	0	46	23.9
La Concordia	0	0	1	0	0	0	0	1	0.0	0	0	1	0	0	0	0	1	0.0
La Ceiba	0	0	0	0	0	0	0	0	0.0	1	0	1	1	0	0	0	3	33.3
Totals	56	2	4	0	2	1	0	65	86.2	12	10	20	7	1	1	0	51	23.5
										Artificial pit-shelters								
										36	40	50	6	1	1	3	137	26.3
El Plan										77	42	93	100	10	10	6	338	22.8
El Retiro										7	21	10	4	1	0	1	44	15.9
La Concordia										0	1	1	0	0	0	0	2	0.0
La Ceiba										120	104	154	110	12	11	10	521	23.0
Totals																		

†Human blood proportion or per cent of engorged females containing human blood.

Table 3. Hosts for bloodmeals of *Anopheles pseudopunctipennis* females from indoor and outdoor resting collections. Data were pooled by each year over all villages and months. The resting collections were conducted in four villages in the foothills near Tapachula, Mexico during the dry season of 1990 and 1991.

	% Blood meal				
Hosts	Indoor[a]	Outdoor (Nat. shelters).	Outdoor (Pit shelters)	Weighted mean[c]	Unweighted mean[d]
1990					
Human	53.8	18.6	-	34.0 [f]	36.2 [f]
Animal[b]	46.2	81.4	-	66.0	63.8
	100 (67)[e]	100 (80)		100 (147)	100 (147)
Horse	19.4	8.8	-	13.6	14.1
Dog	11.9	51.3	-	33.3	31.6
Pig	13.4	16.3	-	15.0	14.9
Chicken	1.5	2.5	-	2.0	2.0
Mixed	0.0	0.0	-	0.0	0.0
Not Ident.	0.0	2.5	-	2.1	1.3
1991	% Blood meal				
	Indoor[a]	Outdoor (Nat. shelters).	Outdoor (Pit shelters)	Weighted mean[c]	Unweighted mean[d]
Human	86.1	23.6	23.0	29.5 [f]	44.2 [f]
Animal	13.9	76.4	77.0	70.5	55.8
	100 (65)[e]	100 (51)	100 (521)	100 (637)	100 (637)
Horse	3.1	19.6	20.0	18.2	14.2
Dog	6.2	39.2	29.6	27.9	25.0
Pig	0.0	13.7	21.1	18.4	11.6
Chicken	3.1	2.0	2.3	2.4	2.5
Mixed	1.5	2.0	2.1	2.0	1.9
Not Ident.	0.0	0.0	1.9	1.6	0.6

[a]Per cent of blood meals by type of host. Blood meals were identified by ELISA.

[b]All animals other than humans plus "not identified" meals combined.

[c]Weighted or crude mean:

(Numbers with human blood indoors + numbers with human blood outdoors)/(total numbers engorged indoors + total numbers engorged outdoors).

[d]Unweighted mean: (Indoor per cent with human blood + outdoor per cent with human blood)/number of pooled samples; *i.e.*, two for 1990 and three for 1991.

[e]Number of specimens tested for blood identification.

[f]Human Blood Index (HBI).

Table 4. (FR)[c] estimates for *Anopheles pseudopunctipennis* females during two years of collections in the foothills near Tapachula, Mexico during 1990 and 1991.

Hosts	% Host Population[a]	% Host Population[b]	1990 % Bloodmeals[d]	1990 FR[a,c]	1990 FR[b,c]	1991 % Bloodmeals[d]	1991 FR[a,c]	1991 FR[b,c]
Human	34.9	76.1	34.0	1.0	0.4	29.5	0.8	0.4
Animal[e]	65.1	23.9	66.0	1.0	2.8	70.5	1.1	2.9
Totals	100	100	100			100		
Horse	0.4	0.9	13.6	34.0	15.6	18.2	45.5	20.9
Dog	9.4	20.5	33.3	3.5	1.6	27.9	3.0	1.4
Pig	1.2	2.6	15.0	12.5	5.8	18.4	15.3	7.2
Chicken	54.2	-	2.0	0.0	-	2.4	0.0	-
Sample size	3753	1719	147			637		

[a]Chicken population included.
[b]Chicken population was deleted.
[c]Forrage ratio = Per cent host blood meals/Per cent host population
[d]Weighted HBI, see Table 3
[e]All animals other than humans plus not identified meals combined

CHAPTER 4

Bionomics of *Anopheles pseudopunctipennis* in the Tapachula foothills area of southern Mexico. I. Adult populations

INTRODUCTION

Malaria is currently the most widely-distributed vector-borne disease in Mexico. The increasing numbers of cases during the last 20 years were interrupted in 1986 by a national program conducted by the Mexican Secretariat of Health. While malaria is still endemic in 29 of 32 mexican states, the number of reported cases went from 101,204 in 1989 to 44% fewer cases in 1990 (Dirección General de Epidemiología-Secretaria de Salud, 1991).

Cases of human malaria occur throughout the costal plains and inland areas of northwestern and southern Mexico. Most cases of malaria in Mexico are caused by *Plasmodium vivax* parasites (Rodriguez and Loyola, 1989). *Anopheles albimanus* is the primary vector on the coastal plain, whereas *An. pseudopunctipennis* is the more important vector at higher elevations, and in drier, inland environments (Hoffman, 1932). *Anopheles pseudopunctipennis* is also known as an important vector in other countries, such as Bolivia, Guatemala, Ecuador, Peru and Argentina (Pan American Health Organization, 1991).

Anopheles pseudopunctipennis is found in mountainous and hilly regions from the southern United States to northern Argentina. Within this geographical range, five subspecies and one variety have been described (Knight and Stone, 1977). Not surprisingly, this species is thought to comprise a complex of sibling species (Baker, *et al.*, 1965).

This species is known to be highly anthropophilic, with the per cent of indoor resting females containing human blood as high as 67% in central Mexico (Vargas, 1938) and above 53% in the Tapachula area of southern Mexico (Chapter 3). However, recent studies have shown that applications of DDT to house walls result in a great reduction of indoor feeding and reduced man-vector contact (Loyola, *et al.*, 1991). This behavior might be described as insecticide-induced exophagic behavior. *Anopheles pseudopunctipennis* is the primary malaria vector in nearly two-thirds of the total malarious area of Mexico (Rodriguez and Loyola, 1989). Some components of *An. pseudopunctipennis* behavior possibly contribute to the persistence of malaria in certain areas of Mexico; consequently, further research on vector behavior might provide improved control of *An. pseudopunctipennis*-transmitted malaria.

Included in this report are results of studies on the dynamics of *An. pseudopunctipennis* adult populations. Data are presented on age structure of natural populations, biting behavior, the effects of macroclimate on biting activity, and the relative efficiency of different sampling methods. Additionally, data are presented on malarial infection rates in wild-caught *An. pseudopunctipennis* populations.

MATERIAL AND METHODS

Geographical and weather description

The study sites are located within the foothills of the Sierra Madre mountain range, about 30 km from the city of Tapachula. This city is located in Chiapas, the southernmost state of Mexico. Tapachula is situated in a transition area between two major ecological zones, the Pacific Coastal Plain to the southwest and the Sierra Madre mountain range to the northeast. Regional weather is tropical with well-defined wet and dry seasons, from May to November and December to April, respectively. Peak malaria transmission occurs in the foothills during the dry season, which corresponds to peak abundance of *An. pseudopunctipennis* populations. Average temperature is 25°C, with relative humidities above 80%. Study sites consisted of four villages at 350-650 m above sea level. Topography of the study area varies from medium-sized hills to very high and steep mountains. Most of the original decidiuous forest has been replaced by intensive coffee cultivation.

During the dry season the main *An. pseudopunctipennis* breeding sites are pools with filamentous algae in mountain streams and rivers. A few large permanent ground pools also serve as breeding sites. Study sites included in this report were located near the margins of the Coatan River (Fig.1), where breeding sites flourish during the dry season. With the beginning of the wet season, however, larval habitats in the river are swept away and densities of *An. pseudopunctipennis* populations quickly decline.

Malaria situation

Vivax malaria is most prevalent in the study area during the drier months of the year. The foothill study sites consisted of four villages, El Plan, El Retiro, La Ceiba and La Concordia, each separated by about 5 km. Cases of vivax malaria have been reported from these villages for the last seven years (Loyola, *et al.*, unpublished data). Transmission months encompass the whole dry season, from late-December to mid-May.

Based on malaria and vector surveys conducted in 1988, the four study villages were characterized with higher or lower risks of malaria transmission. Malaria incidence rates for two higher-risk villages were 39% for El Plan and 12.3% for El Retiro, with 446 and 341 persons living in 74 and 57 houses, respectively. Houses were loosely scattered within these villages, often with more that 100 m separating one house from another. The two lower-risk villages were La Ceiba (>1.0%) and La Concordia (6.7%), with 236 and 240 inhabitants living in 67 and 52 dwellings, respectively. Houses in the latter villages were arrayed along the main road and were located in close proximity to each other, often with less than 20 m

separating one house from another.

Mosquito collections

Studies included in this report were initiated in January 1990 and completed in June 1991, covering 2 dry seasons. Mosquito collections were conducted during one week per village per month. The weekly effort consisted of four nights of 14-hour collections (1700 to 0700) during January, February and March. These three months corresponded to longer periods of darkness. During the other months of surveillance, the collections were conducted hourly for only 12 hours (1800-0600). Mosquito collections were performed by two teams of four collectors per team. Each team worked a six to seven-hour shift each night.

Three types of collection methods were employed: landing collections from exposed legs of the collectors, horse-baited trap collections, and updraft ultraviolet light (UVL) trap collections. For landing collections, a collector used an oral aspirator and flashlight to catch females mosquitoes as they attempted to feed after landing on his exposed feet and ankles. The routine program consisted of performing landing collections simultaneously in three sites (one collector per site): one collector inside of a house, one within five to ten m of the house, and one at a social activity site within the village; *e.g.*, a group gathered along the road, soccer field, *etc.* The latter-type of collection was defined as extradomiciliary. Landing collections were conducted during 50 minutes each hour, followed by a ten-minute break. Teams of collectors were changed at midnight, and starting times of teams were rotated each night. Additionally, individual team members changed collecting sites each night to avoid sampling bias.

The horse-baited trap was located in the center of the village. The collection was performed by tying a horse inside a 4 x 4 x 4-m nylon-screened trap. The bottom of the screening was suspended 30 cm above the ground, and mosquitoes entered the trap through this opening. The field team supervisor entered the trap each hour and used an oral aspirator to collect all engorged and unengorged *An. pseudopunctipennis* females resting on the inner sides of the screening.

Since the updraft UVL trap has been used to sample populations of other anophelines (Sexton, *et al.*, 1986), paired indoor-outdoor trap collections were conducted to determine its efficiency as a sampling device for *An. pseudopunctipennis* females. The traps were placed, one inside and one outside of a house that was at least 300 m from the horse-baited trap and 300 m from the sites where landing collections were being performed. Cages on the light traps were emptied at hourly intervals throughout the night. Fresh-caught mosquitoes were placed in a 0.47-liter cardboard container and provided with a small cotton pad soaked in a

10% sugar water solution.

In addition to the above sampling methods, pit-shelters (Service, 1976) were dug (10 shelters per village) and sampled during the second year of studies. The pit-shelters were 1.5 m deep by 1 x 1.2 m in size and were sampled each morning from December 1990 to May 1991.

All anophelines were identified, and numbers were recorded for each species per hourly collection. Outside ambient temperature was recorded each hour using a metereograph (Qualimetrics, Inc.), along with observations on moon phase. Each morning the collections were taken to the Malaria Research Center in Tapachula, where females were dissected and ovarian tracheoles were examined for parity determinations (Detinova, 1962).

CS-protein rates

Each wild-caught female was dissected to determine parity, the head-thorax was removed for further testing, placed in a 1.5-ml tube, and stored in a container with silica-gel. The head-thoraces were individually assayed by the ELISA for *P. vivax* predominant (VK210) and variant (VK247) CS proteins (Rosenberg, *et al.*, 1989), using monoclonal antibodies NSV-3 (Wirtz, *et al.*, 1985) and 182.1G12 (Wirtz, *et al.*, unpublished data), respectively. The lower detection limits of these assays are approximately 25 and 50 sporozoites, respectively (Wirtz, *et al.*, unpublished data). Unfed, laboratory-reared *An. albimanus* served as negative controls. Use of *An. albimanus* as controls was based on preliminary trials which showed that absorbance values for *An. albimanus* specimens were not noticeably different from absorbance values for wild-caught *An. pseudopunctipennis* specimens. The cut-off value for a positive test was two times the mean absorbance value for seven negative controls. Confirmational tests were performed on initially positive wells and on wells showing suspicious absorbance values. Specimens collected in pit-shelters also were tested for CS protein.

Analyses

Chi-square analyses were employed to compare sample sizes between villages, sample sites, intervals of time during the night, and years. The Kolmogorov-Smirnov test (Siegel, 1956) was used to compare patterns of host-seeking activities during the night. Linear regression analyses were employed (SYSTAT, 1989) to test the effects of temperature and moon phase on host-seeking activities and to determine associations of parity rates with progression of time during the night. The Fisher PLSD tests were performed to compare

parity rates during different quarters of the night.

Monthly survival estimates were calculated for indoor and outdoor populations collected during the malaria transmission months in both years. The probability of daily survival, based on monthly parity rates, was computed with Davidson's (1954) formula. The third root, which equates to the number of days required for completing the gonotrophic cycle (Chapter 2), was employed in these calculations. An estimation was also made of the probability of females surviving for nine days, calculated by raising the daily survival value to an exponent of nine (MacDonald, 1973).

RESULTS

Population dynamics and seasonal changes

In addition to *An. pseudopunctipennis*, *An. albimanus*, *An. apicimacula* and *An. eiseni* were also captured in the four villages. However, the latter three species were captured infrequently and accounted for less than 1% of the total number of anophelines collected during the two years of surveillance.

Seasonal densities of *An. pseudopunctipennis* mosquitoes were analyzed by pooling all collections from all villages (Fig.1). Highest densities were associated with the drier months of the year. In 1990 and 1991, peak densities occurred in January, but population densities subsequently declined. Vector populations in 1991 started increasing three months later, but heavy rains in April eliminated the breeding sites in the Coatan River. After these rains the vector populations largely disappeared until the beginning of the dry season in November.

With pooled collection data for all villages, highest vector densities occurred during January to March in the first year, and from December to March in the second year of surveillance. The numbers of mosquitoes captured per man per night (m/m/n) inside houses for both years were greater in El Retiro and El Plan than in La Concordia and La Ceiba. Range limits for densities in the former two villages during January, February and March of 1990 were 18-27 and 5-23, respectively. Range limits for the same villages during the dry season of 1991, from December to March, were 5-18 and 9-20 m/m/n, respectively. Range limits of anopheline densities for La Concordia were 2-9 in 1990 and 2-3 m/m/n in 1991, and in La Ceiba, the range limits of densities were 1-4 in 1990 and 1-3 m/m/n in 1991 (Table 1). Only El Plan presented indoor densities of *An. pseudopunctipennis* populations that were significantly different in 1991 from population densities of 1990 (X^2=18.67, P<0.01,df=5).

In paired collections, greater numbers of *An. pseudopunctipennis* were captured per man per night outside than inside houses in El Retiro, La Ceiba and La Concordia (Table 1); and greater numbers were collected in extradomiciliary collections than inside houses. However, in El Plan the numbers collected in outdoors (Wilcoxon paired test, P>0.05) or extradomiciliary (P>0.05) collections were not statistically different from indoor collections. In comparisons of outdoor collections from El Retiro and El Plan, the extradomiciliary landing collections were not significantly different from numbers collected within 5-10 m of the houses.

Biting activity

The generalized pattern of nightly host-seeking activity was plotted by percentages of *An. pseudopunctipennis* collected per hour over the total nights collection. The pattern is derived from data pooled from 126 nights of collecting effort. The generalized indoor activity cycle showed a bimodal pattern (Fig. 2), with an early, small peak around 2000 h followed by a second, higher peak at 0100 h. Numbers collected were continually increasing during the time interval from the first to the second activity peaks. In a similar analysis of data from collections conducted outdoors, there was a small peak at 2200 h and a large peak of activity at 2400 h. Conversely, host-seeking activity exhibited in extradomicilary landing collections and animal trap collections peaked at 2100 h and 1900 h, with smaller peaks at 0100 h and 2400 h, respectively. Comparisons of activity patterns, as described, with the Kolmogorov-Smirnov test (Siegel, 1956) revealed that the generalized pattern of indoor activity was significantly different ($P< 0.05$) from patterns exhibited in the outdoor landing collections and horse-baited trap collections. However, there were no significant differences ($P> 0.05$) between patterns of outdoor and extradomiciliary host-seeking activity.

Pooled data from landing collections conducted near the house and at extradomiciliary sites were used to analyze the effects of ambient temperatures and moon phases on host-seeking activity cycles. Data were pooled from a total of 111 nights of collections and sorted into three classes according to mean overnight temperatures. The classes consisted of nights with mean temperatures above 20 °C, from 15 to 20 °C, and below 15 °C. Hourly biting densities were transformed to percentages, plotted versus time and analyzed by simple linear regression (Zar, 1984).

Of 111 nights of observations, the mean temperature was below 15°C during 57 nights (51.3%) and below 20 °C during 99 nights (cumulative value of 89.2%). The record low mean temperature (9.7 °C) occurred in January and the record high mean temperature occurred in April (21.3 °C). Only low temperatures seemed to exert a regulatory effect on *An. pseudopunctipennis* host-seeking activity (Fig. 3). Activity patterns during nights with mean temperatures below 15 °C were characterized by higher peaks of activity during the warmer, earlier hours of the night. This period of peak activity was followed by a trend of diminishing activity during the rest of the night. However, a small, late-night peak of activity was still detectable. During nights with mean temperature below 15 °C, there were significantly fewer (X^2=106.25, P<0.01, df=1) mosquito feeding during the second half of the nights than during those nights with mean temperatures above 20 °C (SYSTAT, 1989). Mean ambient temperatures of 15-20 °C were associated with a unimodal pattern, with peak activity occurring at 2400 h. During nights with mean temperatures above 20 °C, a small surge of activity occurred at 1800 h. This was followed by a large peak of activity at 0200 h.

The effects of moonlight on *An. pseudopunctipennis* activity were analyzed by compiling moon-phase data for all days for which we had outdoor mosquito density data, with population densities of at least one female per hour. The mosquito landing rates were pooled for each hour, averaged, and plotted versus moon phases (Fig. 4). Peaks in landing-rate densities shifted from early to late night as the moon advanced from New Moon to the Last Quarter. This occurred concurrently with the moon's rising later at night. Peaks in landing rates occurred at 1900 and 2100 h during the New Moon, with almost no activity during the second half of the night. Most mosquito activity occurred in the first half of the night during the First Quarter moon phase. Peaks in activity were at 2100 and 2400 h. A unimodal late night peak of activity occurred at 2400 h during the Full Moon phase, and most specimens were captured during the second half of the night. Activity patterns in the Last Quarter moon phase seemed intermediate between activity patterns with late (Full Moon) or early evening (New Moon and First Quarter moon phases) peaks in host-seeking activity (Fig. 4).

Kolmogorv-Smirnov two-sample tests using the cumulative frequency distribution of the sample data, showed no significant differences in night activity patterns between First Quarter to Full and Last Moon, and Full to Last Quarter moon phases. Activity patterns between New Moon to First, Full and Last Quarter phases were significantly different ($P<0.05$). These relationships of moon phase to mosquito activities from two years of data were confirmed by separate analysis of daily mosquito captures on a whole lunar cycle, encompassing one month, as shown in Table 2 and Fig. 4.

Population age-structure

Data analyses were performed to compare the distribution, by physiological condition, of host-seeking *An. pseudopunctipennis* females throughout the night. Of 810 *An. pseudopunctipennis* females captured inside of houses, more than 66% of the 140 specimens captured in the early evening (1800-2100 h) were nulliparous. In contrast, less than 55% of the 153 specimens captured later in the night (0300-0600) were nulliparous (Table 3). Division of total night collections in quarters and comparative parity analysis by Fisher PLSD test, showed significant differences between the earlier versus the late quarter ($P<0.05$). The same nocturnal distribution of host-seeking populations by physiological condition was not observed for host-seeking populations collected outside the houses.

The separate parity rates of specimens collected in landing captures inside and outside of houses were used to calculate daily survival rates of *An. pseudopunctipennis* females per month for January, February, March and April. Only data from El Retiro and El Plan were

used in these calculations. Daily survival rates were then employed to estimate monthly probabilities of surviving for nine days. In 1990, the maximum probability of *An. pseudopunctipennis* females surviving for nine days, based on indoor collections in El Plan, was 25.7% for the month of January (Table 4), while the maximum probability for 1991 was 10.6% during January. Maximum nine-day survival for indoor collected females in El Retiro was 8.4% in February 1990 and 9.5% in January 1991 (the 100% survival rate in March represented only five females). Similar nine-day survival probabilities were calculated on parity rates from specimens collected outside. The maximum monthly probabilities were 35.0% and 18.2% for January 1990 and 1991 in El Plan, respectively. A lower maximum survival rate was determined in El Retiro outdoor populations; i. e., 9.5% in January 1990 and 5.8% during the same month in 1991 (again, the high value of February 1990 was not considered because of the small sample size). The probabilities of survival decreased markedly after the months of peak survival rates for both indoor and outdoor collections during both years (Table 4). The drop in survival rates concomitantly with a drop in adult biting populations corresponded, in general, with the time that houses were sprayed with insecticides in 1990 (April) and 1991 (late January and early February).

Trapping method comparisons

Data were compiled to compare the relative attractivenesss of humans (in different environmental settings) versus horses to *An. pseudopunctipennis* females. Additionally, the efficiency of captures employing the above hosts were compared with ultraviolet light trap captures. During the 18 months of sampling, this sampling scheme was conducted continuously during 286 nights in the four villages. The overall ratio of numbers of *An. pseudopunctipennis* attracted to a single human host versus numbers attracted to a horse tethered inside a screened trap was 1:4 (1266:5476) (Table 5). The trends of numbers collected in landing captures on the human host versus horse-baited traps were similar throughout.

In paired collections of mosquitoes landing on the human host, collections conducted indoors accounted for 21.7% of the total number collected. Collections conducted outside, near the house, accounted for another 35.5%, and collections at the extradomiciliary site accounted for the remaining 42.8% of the total number collected.

Only 113 specimens, mostly females, of *An. pseudopunctipennis* were captured in the ultraviolet light traps during the two sampling years. The highest capture record was four anophelines/trap/night in January.

CS-protein rates

A total of 9,386 head-thoraces of *An. pseudopunctipennis* were assayed for the presence of *P. vivax* VK210 and VK247. The VK210 CS-protein was detected and confirmed in four *An. pseudopunctipennis* head-thorax samples, for an infection rate of 0.0004. The VK247 CS protein was detected and confirmed in six specimens, for an infection rate of 0.0006. The overall infection rate for both CS proteins was 0.001.

The four VK-210 positive specimens were captured in El Retiro and El Plan: one in El Retiro during 1990 (1/2482=0.0004), two (2/2392=0.0008) in 1991, and one in El Plan (1/1837=0.0005) in 1991. Three of these isolates were from the horse-baited trap and one was from a pit-shelter collection. Of the six VK-247 positive specimens, four (4/2392=0.002) were from El Retiro (in 1991) and two were from El Plan: one (1/859=0.001) in 1990 and one (1/1837=0.0005) in 1991. Two of the positive specimens were from horse-baited traps and four were from pit-shelter collections. Combined classic and variant *P. vivax* CS-proteins accounted for infection rates in 1990 of 0.001(1/859) for El Plan and 0.0004 (1/2482) at El Retiro, while combined rates in 1991 where 0.001 (2/1837) for El Plan, and 0.002 in El Retiro (2/2392). No specimens collected in La Ceiba or La Concordia were positive for the *P. vivax* CS proteins.

The appearance of specimens positive for the CS-antigen was associated with times of peak abundance of the vector populations. These times also corresponded to the times of peak malaria transmission in the study area (Loyola, 1991, personal communication).

DISCUSSION

The phenology of *An. pseudopunctipennis* populations in the Tapachula area is strongly associated with seasonal dry season conditions encountered in the mountain and foothill environments. Local populations of this anopheline flourish at higher elevations (above 200 m), with relatively cool ambient temperatures and short dry seasons. According to Shannon (1930) and Hackett (1945), similar environmental conditions characterize the distribution of *An. pseudopunctipennis* populations in Peru and Argentina.

Anopheles pseudopunctipennis is responsible for transmitting malaria in much of Central America and within the Andean region, from northern Argentina to Colombia (Pan American Health Organization, 1991). Throughout the malaria endemic countries of the Andean Region and Central America, populations of this important vector species are largely restricted to the dry season. This consistent pattern is associated with growths of filamentous algae in streams and pools formed in drying rivers (Hackett, 1945). Thus, an abundance of pools with filamentous algae allow a proliferation of *An. pseudopunctipennis* populations during the dry season. As in southern Mexico, the breeding sites are flushed by heavy rains of the wet season, and the rains are followed by a dramatic drop in densities of adult populations.

Regarding the anthropophilic habit of the vector, man-vector contact was higher in the villages at higher elevations and closer to the Coatan River; *viz.*, El Retiro and La Concordia. These villages have also presented higher malaria rates than La Ceiba and La Concordia (Loyola, 1988, unpublished data). Densities of *An. pseudopunctipennis* collected indoors reached levels as high as 23-27 mosquitoes/man/night about one month after the beginning of the dry season during both years of collections. Even when population densities were reduced in 1991 by spraying house walls with DDT, malaria outbreaks occurred in the villages (Malaria Research Center, unpublished data). Loyola, *et al.* (1990) have shown that there is a repellent effect of DDT which results in reduced numbers of *An. pseudopunctipennis* entering sprayed houses in northwest Mexico. As described by Loyola, *et al.* (1991), malaria persists in villages, as in those of the Tapachula foothill area that are routinely sprayed with DDT. Persistence of malaria transmission in treated villages raises the possibility that *An. pseudopunctipennis* has become physiologically or ethologically resistant to DDT. However, the results of outdoor collections near houses and away from houses (extradomiciliary) indicate that the anophelines will opportunistically feed on humans throughout the night. Consequently, malaria might be transmitted by opportunistic feeding outdoors as a result of certain schedules and types of human activities outside.

Interestingly, collections at each of the three sites in El Plan, indoors, outside near the house, and outside away from houses, were similar and were indicative of a strong anthropophilic feeding behavior. In a separate study of *An. pseudopunctipennis* feeding patterns (Chapter 3), 56.7-88.1% of females resting indoors in El Plan had fed on human blood. Coincidentally, man was the more abundant and available host in El Plan.

The feeding behavior of anophelines is controlled by endogenous circadian rhythms, which can be influenced by external factors such as temperature, relative humidity and light intensity (Clements, 1963). Landing collections of *An. pseudopunctipennis* females conducted in the four villages in the Tapachula foothills were designed to document the general host-seeking patterns, as well as activity patterns for different temperatures and different moon phases. Such information should be valuable for designing future studies. In fact, knowledge of host-seeking activity patterns should be useful in designing feasibility studies for use of insecticide/repellent impregnated bednets in malaria control programs.

The cycle of indoor host-seeking activity was bimodal, and while it was similar to the pattern of biting activity for *An. pseudopunctipennis* in Peru, it was different from the unimodal pattern for the same species in Ecuador (Elliot, 1972). The peak at 2000 h was similar to findings from Peru, while the late-night peak occurred three hours earlier than patterns reported from South America. The first peak of activity occurred around the time when residents could be expected to be eating dinner or else be outside chatting with neighbors. The late peak occurred while most residents are sleeping and, as stated by Gillies (1988), " ...a sleeping or reclining host is less easily disturbed by bites and feeding success will tend to be higher." The more efficient vectors, such as *An. gambiae* and *An. funestus*, feed late at night while the human hosts are sleeping (Gillies, 1988).

Populations of *An. albimanus*, the other vector in Chiapas state, are much more abundant in domestic, coastal environments (Bown, *et al.*, 1987) than are populations of *An. pseudopunctipennis* in domestic foothill environments. The former species is crepuscular in its host-seeking behavior (Elliot, 1972) and seems to be an effective malaria vector only when population densities are high (Rodriguez and Loyola, 1989). In contrast, *An. pseudopunctipennis* more frequently feeds late at night, while the human host is asleep, and transmits malaria at much lower vector densities. Although the patterns of host-seeking behavior seem to be important components of vector competence, we do not know the actual contribution these differences make to vector efficiency. Another important aspect of the malaria threat relates to risks imposed by certain types of human behavior. Anthropological studies are currently underway to assess malaria risks as attributes of certain behavioral patterns of the human populations (Birgham, 1990,

unpublished data)

Significantly different bimodal patterns of indoor and outdoor activity were exhibited by the host-seeking populations of *An. pseudopunctipennis*. Differences in timing of peak activity might be explained as a lag of about one hour from from the time mosquito first arrives at outdoor sites to the time it enters the domicile. The one-hour delay from outdoor to indoor peaks of activity was true for both peaks in the bimodal activity patterns.

Most females from the early-evening extradomiciliary and horse-baited trap collections were nulliparous. The early-evening peaks in activity for both types of collections might consist of specimens from dispersal flights and flights of virgin females (Gillies, 1988).

Ambient temperature had demonstrable effects on host-seeking activity. There was a direct regulatory effect at lower temperatures, with a shift in activity to the earlier and warmer periods of the night (Fig. 4). The positive correlation between average night-time temperatures below 15°C and host-seeking is probably linked to the lower temperature threshold for this high elevation species. Low temperature tolerance probably represents a physiological adaptation to survive the cooler climate in high elevations, such as the Sierra Madre mountain range in southern Mexico and the Andean Region in South America (Hackett, 1945). In contrast to the low temperature effects, there was a shift of peak host-seeking activity toward late-night intervals with warmer night-time temperatures. Additionally, higher levels of host-seeking activities were maintained for a greater part of the night. In fact, females continued to feed during most of the second half of the night with warmer temperatures. These findings provide clear evidence of the marked affect ambient temperature can exert on vector behavior.

Activity patterns of host-seeking *An. pseudopunctipennis* populations seemed to be regulated by endogenous circadian rhythms, which were influenced by the lunar cycle. The same lunar periodicity of host-seeking behavior has been documented for *An. dirus* in Bangladesh (Rosenberg and Maheswary, 1982) and *An. farauti* in Papua, New Guinea (Charlwood, *et al.*, 1986). Lunar regulation of activity patterns may occur as a result of cues from visible moonlight. This interpretation is based on the appearance of greater activity during moon-rise, while peak activity occurred when the moon was at its zenith (maximum luminiscence). Host-seeking activity was limited to the early-evening hours during the darker moon phases. Twilight might be the source of some early-evening light that would aid the host-seeking mosquitoes. These behavior patterns closely approximated the host-seeking activity patterns of *An. farauti* in New Guinea, but were slightly different from those of *An. dirus* in India. The competitive effect of moonlight on the results of

mosquito light trapping has been reported by Davies (1975); however, this phenomenon seems unrelated to the interactions of moon phase and host-seeking behavior. Although few studies have been performed, the importance of moon phases to biological systems is emphasized by the finding that mating, emergence and feeding activities of *Clunio marinus*, an intertidal midge, are controlled by lunar and semi-lunar cycles (Page, 1985).

Three methods were employed to collect *An. pseudopunctipennis*. Of the three methods, the inexpensive horse-baited trap was strikingly more efficient at collecting large numbers than either the UVL trap or the landing collections from humans. This reflects the species preference for feeding on larger mammals, such as horse, donkey and cow. Similar preferences have been reported for this species in the Pacific northwestern coast of Mexico (Loyola, *et al.*, 1990), in Peru (Sasse and Hackett, 1950) and also in the Tapachula foothills (Chapter 3). The horse-baited trap is efficient and could be useful in surveying for seasonal population changes and for evaluating control strategies. The landing collections from the collector's feet and hands should continue to be of immense value in epidemiological studies. We found UV light traps to be highly inefficient for samping *An. pseudopunctipennis* populations. However, these traps have been reported to be useful also for sampling *An. albimanus* populations (Wilton, 1975; Sexton, *et al.*, 1986). The former differences could be explained by several factors related to trap light source and design, physiological condition of mosquitoes caught, and moonlight, although there is general agreement that species inhabiting wooded areas, in preference to exposed habitats, appear to be the least attracted to light traps (Service, 1976).

Two important observations were obtained from the assays to detect *P. vivax* CS proteins in wild-caught *An. pseudopunctipennis*. First, our results provide additional evidence that *An. pseudopunctipennis* is a vector of *vivax* malaria in the foothill areas of southern Mexico. Second, our results provide the first evidence that the VK 247 *P.vivax* strain is present in a Mexican vector. However, the ELISA-based results need to be supported with data on salivary gland or gut infection rates from other places in Mexico, simply because there has been a remarkable lack of malaria field studies on *An. pseudopunctipennis*. The latest published work is by Loyola, *et al.* (1991) using a CS-protein ELISA; he reported 0-3.16% VK210 CS protein rates in *An. pseudopunctipennis* from Sinaloa, Mexico. The last known record of sporozoite infection rates in *An. pseudopunctipennis* was from a work in Perú by Hayes, *et al* (1987), who found three out of 117 (1.8%) *An. pseudopunctipennis* females with glands positive for sporozites. Salivary gland infection rates are needed to better quantify the vectorial competence of this species for the malaria parasite. Since the work of Warren, *et al.* (1980), who could not infect laboratory-reared *An. pseudopunctipennis* from a force-mated colony with *P. vivax*

or *P. falciparum*, the vectorial role of *An. pseudopunctipennis* has been controversial.

Our results for the VK247 *P. vivax* strain verifies its presence in Mexico. This finding supports the results of Kain, *et al.* (1992), who found three out of six patients from the foothill region to be positive for the VK247 strain. The results of Kain, *et al.* (1992) were obtained by testing blood dots from dried filter paper with polymerase chain reaction of CS-gene and DNA oligoprobe hybridization, as well as identification of antibody to CS proteins by IFA and ELISA. The VK247 was first isolated in Thailand (Rosenberg, *et al.*, 1989). Antibody to the CS protein VK247 strain also has been reported in Brazil (Cochrane, *et al.*, 1990).

SUMMARY

Field studies on the bionomics of adult *An. pseudopunctipennis* were conducted to assess its relative importance as a primary vector of vivax malaria in southern Mexico. Routine surveillance was conducted in four malaria endemic villages in a foothill region near Tapachula, Mexico. Population densities of *An. pseudopunctipennis* increased during the dry seasons of 1990 and 1991. During both years the outbreaks of malaria occurred in villages at times of peak abundance of *An. pseudopunctipennis* mosquitoes. The pattern of nocturnal host-seeking activity indoors was bimodal. The late night peak in activity was perhaps of greatest epidemiological importance, since it occurred when most residents were asleep and fully exposed to the anophelines. Ambient temperatures below 15 ^{o}C exerted a regulatory effect (reduced activity) on host-seeking activity. Moon phase also exerted control of the pattern of host-seeking activity. Results of enzyme-linked immunosorbent assays performed on wild-caught *An. pseudopunctipennis* specimens documented the presence of natural infections with the classical and new VK 247 *P. vivax* variant. These findings verify the importance of *An. pseudopunctipennis* as a major vector of vivax malaria in Mexico and extend the geographical range of the VK 247 *P. vivax* variant.

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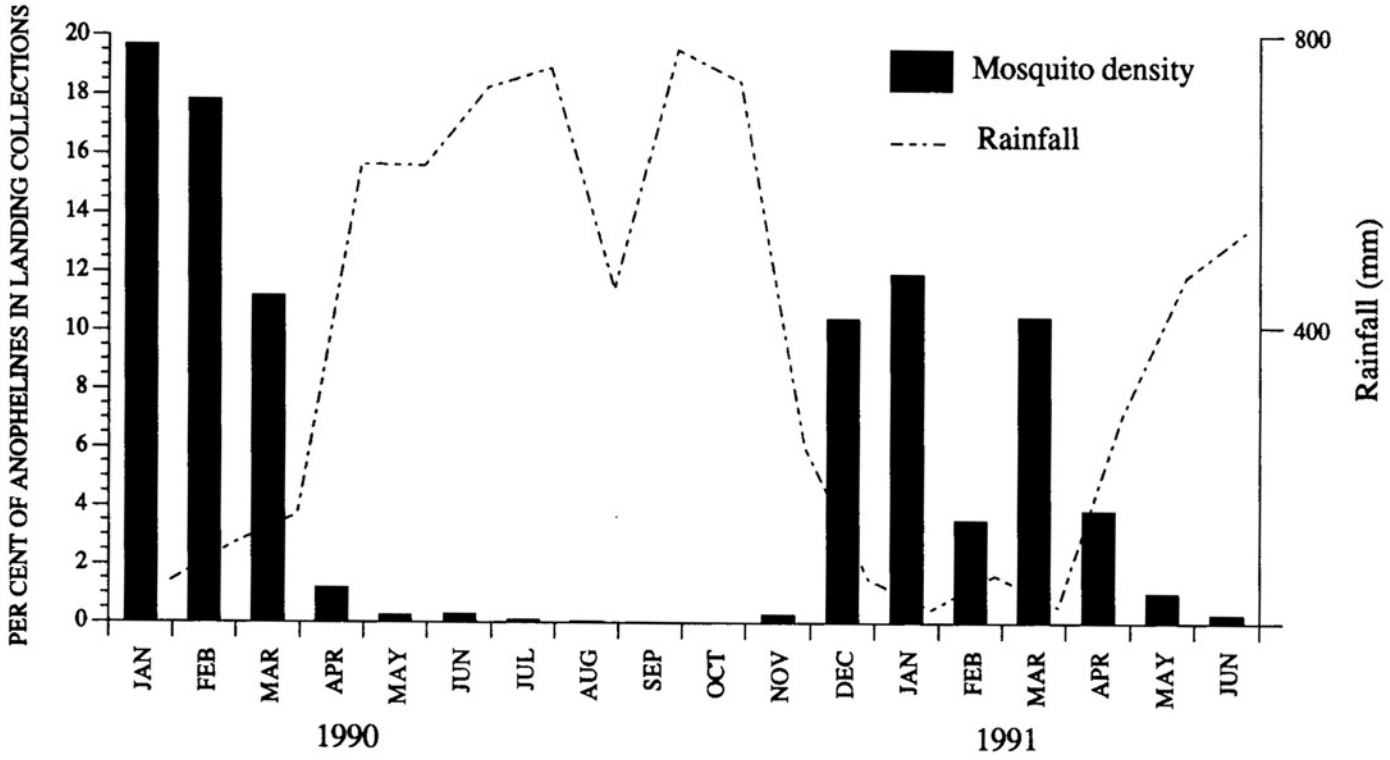

Figure 1. Distribution of rainfall and *An. pseudopunctipennis* populations in the foothills near Tapachula, Mexico from January 1990 to June 1991. Distribution is plotted by monthly percentages of the total numbers collected over all months.

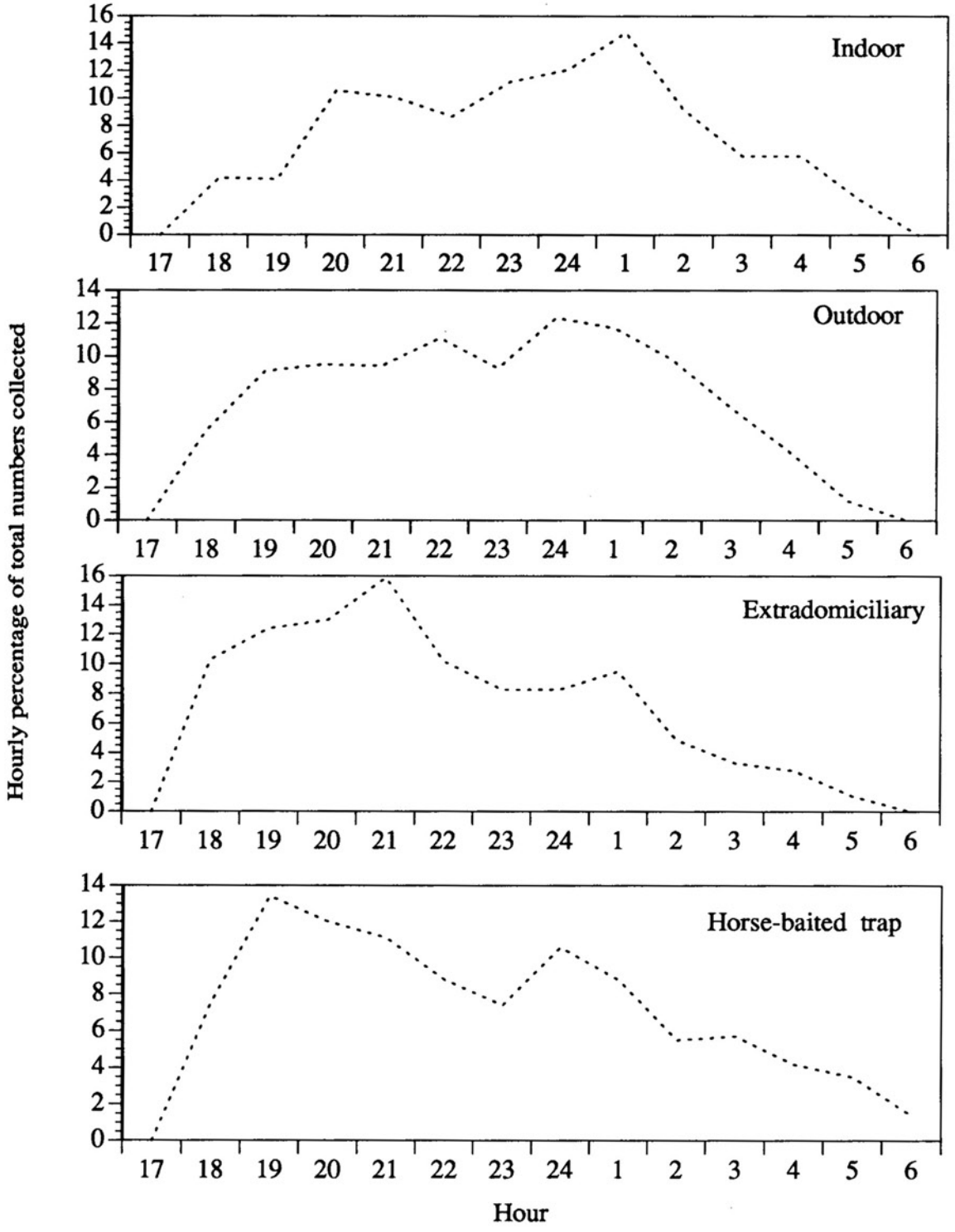

Figure 2. Patterns of host-seeking activities of *Anopheles pseudopunctipennis* females based on 126 nights of collections in four villages in the foothills near Tapachula, Mexico. Data are plotted by percentages of hourly landing collections on human indoors, outdoors near houses and outdoors away from houses (extradomiciliary); and hourly collections from horse-baited trap.

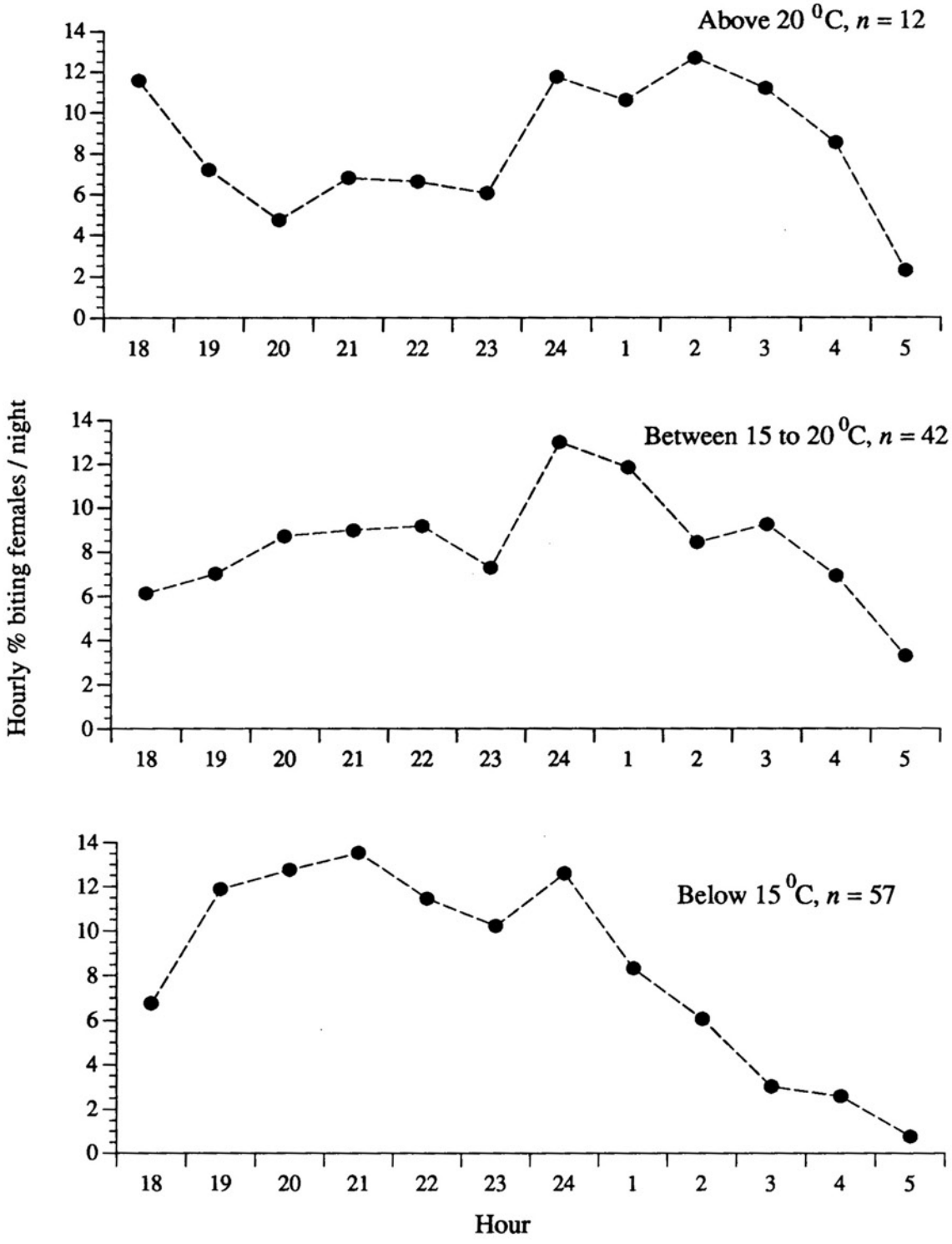

Figure 3. Patterns of host-seeking activity of *Anopheles pseudopunctipennis* females under different conditions of ambient temperature in four villages in the foothills near Tapachula, Mexico. Data are plotted by hourly percenatges of the total numbers collected landing on humans during 1990 and 1991 (n = nights of collections).

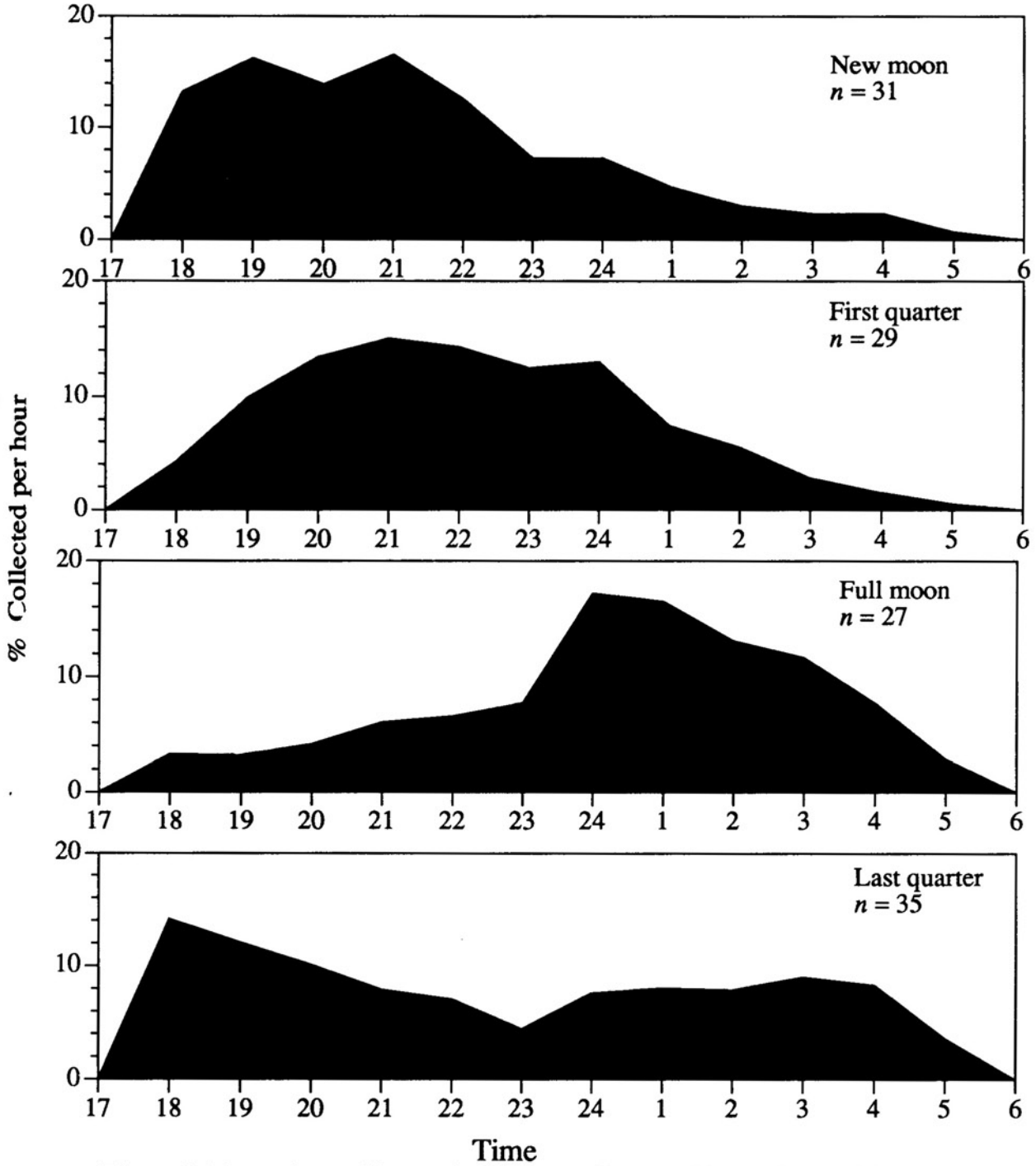

Figure 4. Moon-phase effect on the patterns of host-seeking activity of *Anopheles pseudopunctipennis* females outoors in four villages in the foothills near Tapachula, Mexico. Data were pooled from landing collections on humans during 1990 and 1991. Hourly percentages were based on the total number collected (n = nights of collections).

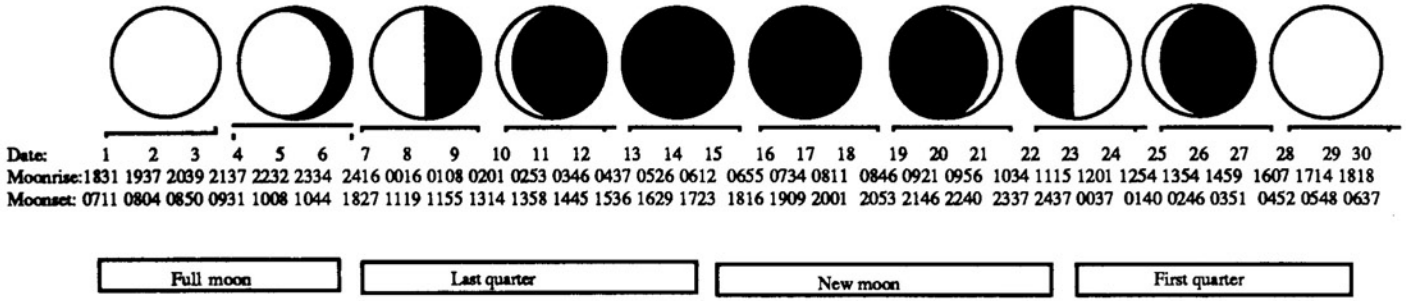

Fig. 5. Phases of moon and times of moonrise and moonset per day at 20° N latitude from 1 January to 30 January, 1991 (Nautical Almanac, 1990).

Table 1. Numbers of *Anopheles pseudopunctipennis* females captured in landing collections indoors, outdoors near houses, and outdoors away from houses (extradomiciliary) in four villages in the foothills near Tapachula, Mexico. Values represent average numbers of mosquitoes per man per night during 1990 and 1991.

		El Retiro			El Plan			La Ceiba			La Concordia		
	Months	*I	O	E	I	O	E	I	O	E	I	O	E
1990	Jan	27	64	49	9	6	9	4	4	5	†-	-	-
	Feb	25	43	34	23	13	14	1	2	1	9	26	23
	Mar	18	30	31	5	2	10	1	<1	2	5	16	14
	Apr	<1	<1	4	0	<1	<1	†-	-	-	2	2	4
	May	<1	<1	<1	0	0	0	0	0	<1	0	2	0
	Jun	0	0	0	0	0	0	0	0	<1	1	1	1
	Jul	0	1<	<1	0	0	0	0	0	0	<1	<1	<1
	Aug	0	0	0	0	0	0	0	0	0	<1	0	0
	Sep	0	0	0	0	0	0	0	0	0	0	0	0
	Oct	0	0	0	0	0	0	0	0	0	0	0	0
	Nov	<1	1	1	0	0	0	0	0	0	<1	<1	<1
	Dec	18	17	77	<1	<1	0	1	1	1	3	2	4
1991	Jan	9	19	47	20	23	11	2	0	0	2	4	4
	Feb	1	2	8	2	8	4	3	3	2	2	1	6
	Mar	5	6	7	9	30	56	<1	0	<1	2	4	6
	Apr	1	1	3	3	7	10	5	9	1	2	3	1
	May	<1	<1	2	<1	<1	2	1	1	1	1	1	2
	Jun	0	0	0	<1	<1	<1	1	0	0	0	0	1

*I = Indoor
O = Outdoor
E = Extradomiciliary
† Not sampled

Table 2. Moon phases and data on host-seeking activity of *An. pseudopunctipennis* females during the month of January 1991. Data derived from collections conducted in four villages in the foothills near Tapachula, Mexico.

	Full moon 1-6 Jan. *n*=3†		Last quarter 7-15 Jan *n*=2		New moon 16-22 Jan. *n*=4		First quarter 23-29 Jan. *n*=3	
Time	Hourly total	Per cent	Hourly total	Per cent	Hourly total	Per cent	Hourly total	Per cent
18:00	9	7.6	8	28.6	10	11.0	7	3.1
19:00	3	2.5	8	28.6	20	22.5	13	5.7
20:00	3	2.5	4	14.3	11	12.4	32	14.0
21:00	2	1.6	1	3.6	19	21.4	35	15.3
22:00	4	3.4	2	7.0	9	10.1	38	16.6
23:00	7	6.0	1	3.6	3	3.4	37	16.1
24:00	29	24.6	1	3.6	7	8.0	35	15.3
01:00	19	16.0	2	7.1	3	3.4	14	6.1
02:00	14	12.0	0	0.0	4	4.5	9	4.0
03:00	12	10.2	0	0.0	1	1.1	6	2.6
04:00	12	10.2	1	3.6	2	2.2	2	0.8
05:00	4	3.4	0	0.0	0	0.0	1	0.4
Totals	118	100	28	100	89	100	229	100

† Number of full-night collections.

Table 3. Numbers of *Anopheles pseudopunctipennis* females collected, dissected and per cent parous from indoor and outdoor landing collections in four villages in the foothills near Tapachula, Mexico. Data are arrayed by quarters and hourly collection intervals from 117 nights of collecting.

		Indoor population			Outdoor population		
Quarter (Q)	Collection hour	Numbers caught	Numbers dissected	% Parous	Numbers caught	Numbers dissected	% Parous
Q1	18:00-19:00	29	27	33.3	68	24	50.0
	19:00-20:00	58	44	36.4	96	66	44.0
	20:00-21:00	53	39	36.0	102	68	56.0
Q2	21:00-22:00	72	43	51.0	97	71	38.0
	22:00-23:00	71	36	44.4	126	71	41.0
	23:00-24:00	79	47	45.0	109	63	38.0
Q3	24:00-01:00	104	50	40.0	174	75	44.0
	01:00-02:00	107	59	49.0	151	56	46.0
	02:00-03:00	82	41	39.0	129	56	27.0
Q4	03:00-04:00	68	28	50.0	131	48	42.0
	04:00-05:00	59	24	46.0	99	31	29.0
	05:00-06:00	26	12	83.0	36	15	47.0
	Total	810	453		1322	645	

Table 4. Estimates of survival rates of *Anopheles pseudopunctipennis* females in the villages of El Retiro and La Ceiba, located in the foothills near Tapachula, Mexico. Estimates are based on indoor and outdoor landing collections during 1990 and 1991. Data are presented by month on numbers collected, per cent parous, probability of survival for one day and the per cent that will survive for nine days.

Village		Indoors				Outdoors			
El Retiro		*n*	Parity rate (%)	Daily[a] Survival Probability	% Survival 9 days[b]	*n*	Parity rate (%)	Daily Survival Probability	% Survival 9 days
1990	Jan.	16	31.3	0.68	3.0	35	45.7	0.77	9.5
	Feb.	38	44.7	0.76	8.4	55	36.4	0.71	4.6
	Mar.	45	40.0	0.74	4.9	54	42.6	0.75	7.5
	Apr.	1	0	-†	-	2	0.0	-	-
1991	Jan.	31	45.2	0.77	9.5	48	39.6	0.73	5.8
	Feb.	5	100	1.00	100	7	71.4	0.89	35.0
	Mar.	19	37.0	0.72	5.2	24	33.3	0.69	3.5
	Apr.	3	0.0	-	-	2	0.0	-	-
El Plan									
1990	Jan.	39	64.1	0.86	25.7	24	70.8	0.89	35.0
	Feb.	45	40.0	0.74	4.9	27	33.6	0.69	3.5
	Mar.	10	20.0	0.58	0.7	9	22.2	0.61	1.2
	Apr.	-	-	-	-	-	-	-	-
1991	Jan.	45	46.7	0.78	10.6	81	56.8	0.83	18.2
	Feb.	8	25.0	0.63	1.5	32	50.0	0.79	11.9
	Mar.	18	22.2	0.61	1.2	56	8.9	0.45	0.1
	Apr.	-	-	-	-	1	0	-	-

[a] $\sqrt[gc]{\text{Parity rates}}$, gc= 3 days (Davidson, 1954).

[b] Daily probability of survival^9 x 100 (MacDoanld, 1973).

†sample size too small.

Table 5. Total numbers of *Anopheles pseudopunctipennis* females collected in landing collections, horse-baited trap, updraft UVL traps, numbers of full-night collections and hours of collections per night are presented. The numbers per night that were attracted to man (landing collections), horse and light trap are included for the three collecting methods, respectively. All data were derived from collections conducted in four villages in the foothills near Tapachula, Mexico during 1990 and 1991.

1990 Months	†Landing collections	†m/m/n	Horse-baited Trap	m/h/n	††Light Trap	††m/lt/n	Numbers of nights	Hours /night
Jan.	693	21	893	81	40	4	11	12
Feb.	750	18	1359	91	34	2	15	14
Mar.	538	13	576	36	8	1	16	14
Apr.	61	1	111	8	1	<1	14	14
May.	10	<1	5	<1	0	0	19	14
Jun.	9	<1	11	1	0	0	16	12
Jul.	5	<1	14	1	0	0	17	12
Aug.	2	<1	0	0	0	0	18	12
Sep.	0	0	0	0	0	0	16	12
Oct.	0	0	0	0	0	0	17	12
Total	*2068*		*2969*		*83*		*159*	
90-91								
Nov.	13	<1	9	1	0	0	15	12
Dec.	270	8	493	35	8	1	14	12
Jan.	721	17	1119	59	16	1	19	14
Feb.	158	4	257	17	3	<1	15	14
Mar.	304	7	373	27	3	<1	14	14
Apr.	200	5	195	12	0	0	17	14
May.	56	1	56	3	0	0	17	14
Jun.	9	<1	5	<1	0	0	16	12
Total	*1731*		*2507*		*30*		*127*	
TOTALS	3799/3=1266.3		*5476*		*113*		*286*	

†Combined indoor, outdoor and extradomiciliary collections
††Combined indoor and outdoor UV up-draft light traps
m/m/n = mosquitoes/man/night; m/h/night = mosquitoes/horse/night; m/lt/n = mosquitoes/light trap/night.

CHAPTER 5

Bionomics of *Anopheles pseudopunctipennis* in the Tapachula foothills area of southern Mexico. II. Larval populations

INTRODUCTION.

Anopheles pseudopunctipennis Theobald, 1901 is the most widely spread malaria vector in Mexico. This species and the coastal dweller, *An. albimanus*, have been the main targets of the National Malaria Control Program in Mexico during the last two decades (Rodriguez and Loyola, 1989).

Anopheles pseudopunctipennis generally inhabits areas of higher altitudes (over 200 m above sea level) throughout its geographical range. This species is found from the southern United States to northern Argentina and is generally associated with mountain ranges such as the Sierra Madre in Mexico and the Andes in South America (Aitken, 1945). Formation of the larval breeding sites in this rugged topography is regulated by annual local rainfall patterns. When the wet season ends, breeding sites are formed by pools, ponds and lagoons developing in the margins of rivers and streams. Floating plants, grasses or mats of filamentous algae are characteristic of these breeding sites (Hoffmann and Samano, 1938; Savage, *et al.*, 1990). As adults emerge from these sites they readily fly to human settlements, where they feed avidly on humans and large domestic mammals. Consequently, this species is an important vector of *vivax* and *falciparum* malaria in several countries, including Mexico, Guatemala, Honduras, Ecuador, Peru and Bolivia (Pan American Health Organization, 1991).

Despite the usefulness of control measures directed against larvae of malaria vectors, most malaria control programs worldwide have relied heavily on the use of DDT and other residual insecticides to control adult mosquitoes (Service, 1976). Thereby, investigations on the ecology and biology of the larvae of *An. pseudopunctipennis* and other vectors have infrequently been performed in most countries, including Mexico.

Vector biology is an important component of malaria epidemiology. The population dynamics of adults and larvae of vector species impinge on malaria transmission risks and on the success of malaria control programs. Since little is known about *An. pseudopunctipennis* biology, the Malaria Research Center in Tapachula, Mexico, launched a program of *An. pseudopunctipennis* research in 1989. One research goal was to obtain information that might help to develop new approaches to the control of *An. pseudopunctipennis*-transmitted malaria. Data on the gonotrophic cycle, daily survival rates, feeding patterns, and *Plasmodium vivax* circumsporozoite protein rates in adults collected in the Tapachula foothills have been previously reported (Chapter 2, 3 and 4). Included here are observations on the bionomics of the larval stage, including seasonal changes in larval abundance, as well as observations on the seasonal dynamics of larval habitats.

MATERIALS AND METHODS

Study area

The study sites are located in a hilly area near the city of Tapachula. This is also the Soconusco Region, which is part of the West Sierra Madre Mountain range in southern Mexico. Elevations of sampling sites were 300 to 650 m above sea level. The region is mostly devoted to coffee production and, secondarily, to banana and cacao cultivation. Although the natural vegetation is continuously being replaced by coffee plants, remnants of a deciduous tropical forest still exist. The average annual rainfall of 3800 mm is distributed mostly within a six-month wet season, from May to November which is followed by a dry season from December through April. Average annual temperature is 25°C, and relative humidity is 80-90% for most of the year. Topography varies from gentle slopes to very steep hills and mountains, and narrow valleys. A large number of creeks, streams, seepage springs and temporary roadside puddles are present during the wet season. Some of these habitats support anopheline breeding; however, most dry out during the dry season. Of these, only the large ground pools, lagoons, reservoirs, man-made gravel pits, *etc.*, might contain water throughout the year.

The Coatan River is an important topographical feature of malaria epidemiology in the Tapachula foothills. This river is one of the largest in the mountain range, and it cuts across about 16 km of foothill region. Several villages on the banks of the river are experienced seasonal vivax malaria. Four of these villages, El Retiro, El Plan, La Ceiba and La Concordia, were selected as study sites (Fig. 1).

Water of the Coatan River has been used in the hydroelectric plant, "Cecilio de Valle", for the last 20 years. The plant is located about 12 km down from "La Boquilla," which is the point where the water is collected and channeled into a tube leading into a reservoir at the hydroelectric plant (Fig. 1). The water used in the hydroelectric plant is dumped back into the Coatan River at a site near La Concordia village. The environmental conditions created by this diversion of water result in creation of abundant *An. pseudopunctipennis* breeding sites along the margins of the river, from late December to early May, during the dry season.

Wet season breeding sites

During the wet season of 1990, mosquito breeding sites were surveyed throughout the study area, to include villages and coffee plantations. Pools in margins of creeks,

streams, irrigation canals and springs, as well as lagoons, rain puddles, ponds, reservoirs, dams, ground pools and gravel pits were surveyed for the presence of larvae. At each of these sites, larvae were collected with 30 dips, using standard 350-ml dippers (Service, 1976). During the first site visit larvae were collected and taken to the Malaria Research Center insectary where they were reared to adults and identified (Wilkerson *,et al.,* 1990). To prevent possible confusion with anopheline species besides *An. pseudopunctipennis* that might be ovipositing in selected sites, those initially found to harbor other *Anopheles* species were eliminated from the study, and only *An. pseudopunctipennis* and anopheline-negative sites were selected and monitored. Selected sites were visited either monthly or bimonthly during the next 12 months. Larvae were collected, counted and returned to their breeding site to avoid population disturbances that might affect the larval densities.

The wet season habitats of *An. albimanus* and *An. pseudopunctipennis* larvae were classified using criteria established by Rejmankova, *et al.* (1991). Estimates of larval productivity for each class of habitat were based on the per cent of total collections positive for larvae and the mean density of larvae/dip. Additionally, the following statistics were calculated from the surveillance data:

1. Per cent of visits that were positive for larvae over all sampling visits
2. Per cent of visits when a wet season class of habitats had water during the total number of times that such sites were sampled
3. Per cent of visits when habitats of a given class had water during visits in the dry season

Wet season sites were considered productive if more than one specimen was collected and if the site was positive for larvae during more than one visit.

Habitats were described further by collecting and identifying aquatic and semi-aquatic plants from each habitat (Miranda, 1952). Filamentous algae was recorded as present or absent at each site. Temporal associations between larval abundance, algae and plant populations for every class of wet season breeding habitat were analyzed by Fisher exact test, two-tailed test (Zar, 1984).

Dry season breeding sites

Surveillance of transects along the Coatan River was used to quantify numbers of pools and densities of larvae at different localities during the dry season. A transect consisted of a linear distance of 200 m along the river, and both sides of the river were

included in the transect. We were interested in correlating larval production with adult population densities at four villages along the river. Consequently, one transect was located at each of the four villages (Fig. 1). Additionally, densities of adult populations were routinely sampled within each village. Distances between transects and village sites were about one km to El Retiro, 0.5 km to El Plan, two km to La Ceiba and one km to La Concordia.

Sampling of transects began at the end of the wet season, in November 1990, and was continued throughout the dry season, until June 1991. Transects were sampled at two-week to one-month intervals. Larvae were collected with a standard 350-ml dipper, and ten dips were made at each potential breeding site (river pool). This was considered to be an adequate sample size, because the surface area of individual pools was seldom more than 2 m^2. Some field-collected specimens were taken to the Malaria Research Center entomology laboratory for identification, while most larvae were returned immediately to the collection sites.

Changes in larval densities were estimated by mean numbers of larvae per dip. The number of available dry season sites/transect/month was another estimator of malaria vector abundance. The Pearson correlation coefficient and simple linear regression analyses were used to test for associations between densities of larvae and numbers of dry season breeding sites; larval abundance and per cent of surface area with filamentous algae; and larval density versus size of habitat (SYSTAT, 1989). Additionally, the number of larvae/m^2 of filamentous algae was estimated by taking the total surface area covered with algae for all dry season pools of a transect, divided by the total number of larvae collected in the pools.

Usefulness of larval surveillance as an estimator of adult densities in study villages was evaluated by correlation coefficients of monthly densities of larval and adult populations. The numbers of larvae were transformed to Log (x +1) (Service, 1976) for use in the analyses. Data on population densities consisted of pooled indoor and outdoor collections of adult *An. pseudopunctipennis* females as they landed on human hosts; such information was generated by concurrent surveillance of adult populations in the villages (Chapter 4). The numbers of breeding sites in different transects were compared by the non-parametric Wilcoxon rank test (Siegel, 1956).

RESULTS

Wet season breeding sites

A group of 75 mosquito breeding sites was surveyed in the wet season of 1990. Ten sites were identified and eliminated from surveillance as *An. albimanus* and *An. apicimacula* habitats. Almost 70.0% of the wet season sites were transitory, consisting of rain puddles (26.2%), streams (17.0%), seepage springs (12.3%) and pools in river margins (12.3%) (Table 2). These transitory habitats produced more than two-thirds (70.1%) of the total numbers of larvae collected during the wet season. Seepage springs produced the largest numbers of larvae (25.1%), followed by ponds (21.9%), rain puddles (15.2%), river margin pools (15.1%) and streams (14.7%). Lagoons, gravel pits and reservoirs produced relatively few *An. pseudopunctipennis* larvae (Table 1). Although these habitats were most abundant during the wet season, some of the wet season habitats were also available in the dry season; such habitats were continuously monitored during both seasons.

Pools associated with seepage springs and river margins were most frequently positive for *An. pseudopunctipennis* larvae throughout the year (56.6% and 40.0% respectively) (Table 1). These results were based on 12 continuous months of surveillance. Rain puddles and gravel pits were less frequently positive for larvae (22.5% and 13.2%, respectively), (Table 1). Habitat availability from wet to dry season varied drastically for some favored habitats; *e.g.*, seepage springs were available during 83.0% of all wet season visits, but were available for only 11.8% of all dry season visits. Similar reductions in availability were documented for rain puddles (87.4 to 18.0%) and pools in river margins (90.3 to 0.0%) and streams (91.0 to 25.0%). Larger bodies of water, such as ponds, large lagoons and gravel pits, were not heavily affected by seasonal changes, and were available throughout the year.

Larval abundance in the study area was negatively associated with annual rainfall (Fig. 2). Maximum larval abundance occurred during the dry season and was least during the wet season.

Fifty-one (78.5%) of the wet season habitats contained variable densities of *An. pseudopunctipennis* larvae during one or more visits (Table 2). All of eight seepage springs were positive for *An. pseudopunctipennis* larvae one or more times during all visits. Additionally, ten (91.0%) stream, seven (87.5%) river pool and seven (87.5%) lagoon habitats were positive for *An. pseudopunctipennis* larvae during at least one visit (Table 1). Filamentous algae, mostly species of *Spyrogira* and *Oedogonium* (Prescott,

1978), were found in 46.2% of all wet season habitats and 59.0% of the habitats that were positive for larvae (Table 2). Filamentous algae were significantly associated with seepage springs (Fisher exact test, P<0.05). A high percentage (72.7%) of streams also contained algae. Certain types of floating and emergent vegetation were statistically positivelly associated with specific wet season habitats ($P<0.05$). Associations were documented for *Ludwigia octovalvis* and *Panicum* spp. with river margin pools, *Heteranthera limosa* with seepage springs; and *Eichhornia crassipes* and *Pistia stratiotes* with ponds. Weaker associations ($P<0.10$) were found between the presence of *An. pseudopunctipennis* larvae and *Heterantera limosa*, *Ludwigia octovalvis* and *Paspalum* sp. (Fisher exact test, two-tails).

Dry season breeding sites

The tnumbers of pools and patches of filamentous algae along transects of the Coatan River during the dry season were negatively related to rainfall (Fig. 3). The annual rainfall pattern consisted of diminishing rain in November, followed by low amounts of rainfall from December through March. Rainfall started to increase in late April, followed by heavy rains in May. Isolated river pools that served as *An. pseudopunctipennis* larval habitats began to appear in November. The numbers of these habitats continued to increase throughout the dry season; then the habitats were washed away by increased water flow in May. In June, no dry season habitats were present in the transects (Fig. 4).

Overall, the largest numbers of habitats were recorded for transects at El Retiro and El Plan, with means of 56.1 and 57.1 per transect, respectively (Table 3). Fewer habitats formed along the transect at La Ceiba, with a mean of 24.8 pools. The least number of habitats was recorded for the transect at La Concordia, with a mean of 0.7/transect. There were no differences in numbers of habitats between El Retiro and El Plan (Wilcoxon rank test, $P>0.05$), but these sites were statistically different from La Ceiba and La Concordia ($P<0.05$).

Anopheles pseudopunctipennis larvae appeared quickly as habitats formed in the transects. The percentages of such habitats with *An. pseudopunctipennis* larvae varied from 64.4% to 76.6% (Table 4). There was a clear and direct relationship (as defined by linear regression analysis) between the monthly numbers of breeding sites and average densities of larvae for transects at El Retiro (r=0.655, ß=0.015, $P=0.13$), El Plan (r=0.583, ß=0.011, $P=0.036$) and La Ceiba (r=0.555, ß=0.023, $P=0.049$). This

relationship was not detectable with data from La Concordia.

The presence of filamentous algae was an important characteristic of the riverine breeding sites during the dry season (Table 4). Several genera of filamentous algae belonging to the Phylum Chlorophyta grew in the sunny pools. *Spirogyra, Chladophora, Oedogonium* and *Closterium* comprised the four prevalent genera, with two species of the first genus being most frequent; *viz., S. malmeara* and *S. mayuscula* (Prescott, 1978). Filamentous algae were consistently present in the four transects, with the lowest mean per cent of algae and pool associations recorded for La Ceiba (62.5%) and the highest, in La Concordia (83.3%). Representative data from transects at El Plan and El Retiro were used to define a statistically significant ecological relationships between presence of larvae and presence of filamentous algae (X^2=29.825, df=1, P<0.01, n=130). The densities of larvae were positively associated with the amount of surface area covered by filamentous algae (r=0.245, ß=0.282, P=0.05). There was a similar association of larval density with the percentage of total habitat surface area covered with filamentous algae (r=0.438, ß=0.011, P=0.00).

No association was found between the total area of habitats within the transect and densities of larvae. Larger numbers of habitats with filamentous algae occurred from January to March. A mean of 33.0 larvae were collected/m^2 of filamentous algae in El Retiro, compared to means of 28.6, 16.7 and 53.9 for El Plan, La Ceiba and La Concordia, respectively (Table 4).

The average size of habitats (m^2 of surface area) during the dry season was similar for El Retiro, El Plan and La Ceiba, with range limits of 1.1-1.9 m^2. On average, the few habitats in the transect at La Concordia were smaller (Table 4).

Pearson correlation coefficient of monthly densities of larvae versus numbers of *An. pseudopunctipennis* adults were significant only for data derived from El Plan (r=0.678, P<0.05). Nevertheless, the general patterns in population abundance of adults and larvae were similar for each of the villages. In other words, when larval densities were high, densities of adult populations were also high (Fig. 5).

DISCUSSION

A distinct seasonality in types and availability of habitats for larvae of *An. pseudopunctipennis* was documented in twelve months of collection data. During the wet season, 70.1% of the larvae were collected from rainfall-dependent habitats, such as rain puddles, seepage springs, streams and pools in river margins. Smaller numbers of larvae were collected from the long-lived ponds, lagoons and reservoirs (Table 1). As rainfall diminished during the dry season most of the transient water bodies dried out. However there was a concurrent increase in numbers of optimal breeding sites along several kilometers of the Coatan River as the flow of water decreased during the dry season.

Population densities of *An. pseudopunctipennis* larvae and adults, and malaria transmission rates were at peak levels during the dry season (Fig. 5). Such rain-dependent seasonal changes in vector abundance have been documented for other mosquito species and other groups of insects in the non-seasonal tropics (Wolda and Galindo, 1981).

Seepage springs were relatively common in the foothills during the wet season, when they were important breeding sites and produced the largest numbers of larvae. In total, 56.6% of all the sampling visits to seepage spring habitats were positive for *An. pseudopunctipennis* larvae, including both wet and dry seasons (Table 1). This important relationship also has been documented for this anopheline in Oaxaca, Mexico (Hoffmann and Samano, 1938), Peru (Shannon, 1930), Argentina (Shannon and Davis, 1927) and Ecuador (Levi-Castillo, 1945).

We do not know if there is any wet season diapause occurring within the *An. pseudopunctipennis* populations in southern Mexico. Hoffmann (1929) reported collecting overwintering larvae in springs from Central Mexico. Since he found first instar larvae during cooler months, even with freezing temperatures, he agreed with the assessment by Shannon and Davis (1927) that some cool weather breeding occurs simply because of favorable, warmer temperatures in the seepage spring habitats.

Most wet season habitats containing *An. pseudopunctipennis* larvae also contained filamentous algae and other aquatic plants. Filamentous algae of the genera *Spirogyra* and *Oedogonium* (Chlorophyta) were found in 46.2% of the wet season habitats included in the sampling program and were uniformly present in the seepage spring habitats (Table 2). Moreover, filamentous algae grew densely in pools within transects along the Coatan River, with greatest abundance occurring during January to April (Table 4). Savage, *et al.* (1990) reported the *An. pseudopunctipennis*-filamentous algae relationship as a highly predictive characteristic of wet and dry season habitats. Furthermore, this phytoecological

relationship has been reported for *An. pseudopunctipennis* throughout its geographical distribution from Mexico to South America (Aitken, 1945; Hackett, 1945; Shannon and Davis, 1927). Hoffmann and Samano (1938) proposed that the algae served as shelter and food for the larvae; also, he classified winter breeding sites in Oaxaca, Mexico, according to whether *Hydrodictyon* or *Spirogyra* algal species were most prevalent.

Savage, *et al.* (1990) reported that *Heteranthera limosa*, a semi-aquatic plant, was significantly associated with springs in which *An. pseudopunctipennis* larvae were collected. Additionally, floating plants and flooded-but-emerged grasses were found to be strongly associated with some wet season habitats. Examples of these associations were ponds and lagoons with water lettuce, *Eichhornia crassipes,* and the water hyacinth, *Pistia stratiotes*, representing the floating plants, and several genera of grasses, *e.g.*, *Paspalum*, *Cyperus* and *Panicum* representing the flooded-but-emerged grasses. We believe that floating and emerged vegetation, either macrophytes or microphytes, might stimulate *An. pseudopuctipennis* females to select a particular oviposition site. Interestingly, larvae were seldom collected from wet season habitats that contained little or no vegetation; *e.g.*, gravel pits, lagoons and a reservoir (Table 1).

When heavy rains begin at the end of the dry season, the river pools containing filamentous algae and larvae are swept clean by increased river flow. This occurs when densities of adult anopheline populations are high. It seems likely that with the sudden disappearance of preferred oviposition sites, females begin selecting the rain puddles as alternative sites for egg deposition. Intense egg-laying activity as a result of dense adult populations in May (Fig. 2) and increased per cent of rain puddles containing larvae (15.2%) seem to support this assessment (Table 1). Nevertheless, we believe this simply reflects the mosquitoes' ability to select secondary oviposition sites when their preferred habitats are not available.

The transect method was useful for studying the dry season breeding sites of *An. pseudopunctipennis* in the Coatan River. Increases in numbers of habitats and densities of larvae/pool occurred concurrently (Table 3, Fig. 4). The increase in numbers of pools in the river bed was inversely related to rainfall. This is the expected situation, which seems to characterize the seasonal dynamics of *An. pseudopunctipennis* throughout its geographical range (Shannon, 1930). All but one of the transects showed a negative relationship in numbers of pools with decreased rainfall. However, the transect at La Concordia contained fewer pools during the dry season than during the wet season. This surprising result occurred because water is diverted from the river above El Retiro to a hydroelectric plant (Fig. 1). The canal for diverting the water has been in use since 1966. The diversion of water provides a 16-km stretch of river with reduced water flow during

the dry season. All transects and study villages, with the exception of La Concordia, were located along the river where water flow is reduced. Water is returned to the river about 500 m above the La Concordia transect. The hydroelectrical plant discharge results in increased volume and speed of river flow and in decreased numbers of pools in the La Concordia transect. Densities of adult and larval mosquitoes were equally low at La Concordia. This observation supports the idea that effective larval control can be achieved through environmental modifications or manipulations; *e.g.*, draining pools and eliminating pool-forming depressions. These methods were successfully employed to control *An. pseudopunctipennis* in Ecuador (Levi-Castillo, 1945).

The associations of *An. pseudopunctipennis* larvae with habitats containing filamentous algae were stronger in the dry season (Table 4) than in the wet season (Table 2). The prevalent genus of filamentous algae in the dry season was *Spirogyra,* followed by *Cladophora, Closterium* and *Oedogonium.* The microhabitats provided by the algae resulted in larval densities as high as 62 per m^2 of surface area of filamentous algae (Table 4). Range limits for mean numbers of larvae were 16.7 to 53.9 larvae per m^2 of surface area of filamentous algae. Fortunately, the small pools (average size less than 2 m^2) are focalized along the river margins during the transmission season. This placement of breeding sites should greatly facilitate the task of larviciding as a malaria control measure. The strong association of larvae with filamentous algae may provide potential for control in the future through use of herbicides and genetically manipulated algae containing toxic bacteria crystals; *e.g.*, *Bacillus thuringensis israelensis* and *B. sphaericus* (W. H. O., 1987). However, this would require additional research to more clearly define the relationships of algae and *An. pseudopunctipennis* larvae.

There was a correlation in numbers of larvae and numbers of adults collected at El Plan. Correlations between sample sizes of the immature and adult populations were weak for the other three study sites. Regardless, there was general agreement of sample data in population trends (Fig. 5). In other words, population highs and lows were frequently the same, although the magnitude of population sizes was less apparent. Partly, the lack of statistically significant correlations in larvae-adult population densities could be related to the use of insecticides in villages to control the populations of adults. Unfortunately, insecticides for control of adults and larvae were not applied in a uniform manner at each of the study sites. For example, in El Retiro, DDT was sprayed on house walls in January 1991, whereas, focal control was employed in El Plan the same month, based on selectively spraying only those houses where malaria cases were previously reported (Loyola, 1991, unpublished data). This difference in insecticide treatment probably had an impact on the densities of larvae and adult vectors in the two villages

(Fig. 5). In the case of El Retiro, the applications of DDT to all house walls might have resulted in reduced larval population densities. We must consider this possibility simply because the treatment of house walls with DDT was reported to reduce larval densities in rice fields in Central Mexico (Gahan ,*et al.*, 1949). The focal spraying of houses in El Plan probably reduced a small segment of the house-visiting adults, and therefore had a smaller total effect on larval populations.

Other factors accounting for discrepancies in densities of adult versus larval populations in the villages include variations in water flow and differences in distances of study villages from the Coatan River. A sudden release of water into the river in March, while the canal was under repair, eliminated many dry season pools in the transect at El Retiro, as seen in Fig. 5. On the other hand, high densities of larvae in the La Ceiba transect did not correspond to the low densities of adult mosquitoes in the village, probably because the village was almost two km from the river. Although Levi-Castillo (1945) reports that the flight range of *An. pseudopunctipennis* in Ecuador is 3.6 km the two km separating La Ceiba from the River is probably beyond easy flight range for most of the vectors.

SUMMARY

A study on the population dynamics of *Anopheles pseudopunctipennis* larvae was conducted in a foothill region near Tapachula, Mexico. Systematic surveillance of wet season and dry season habitats was conducted from 1990-1991. Sampling along transects of the Coatan River was employed to quantify habitat availability and population densities of larvae during the dry season. During the wet season, larvae were most abundant in temporary habitats, such as seepage springs, rain pools and pools in stream and river margins. The temporary habitats disappeared during the dry season, which occurred concurrent with increasing densities of larvae in habitats within transects along the Coatan River. The great abundance of the dry season riverine habitats; *viz*., small pools with filamentous algae, resulted in peak densities of host-seeking adult populations in villages associated with the river. During both seasons, there were significant associations in the presence and abundance of larvae with habitats containing filamentous algae, and secondarily with aquatic and semi-aquatic plants. There was a significant correlation between mean numbers of larvae per habitat and mean numbers of breeding sites in the transects. Overall, populations of *An. pseudopunctipennis* larvae were very abundant during the dry season and relatively uncommon during the wet season.

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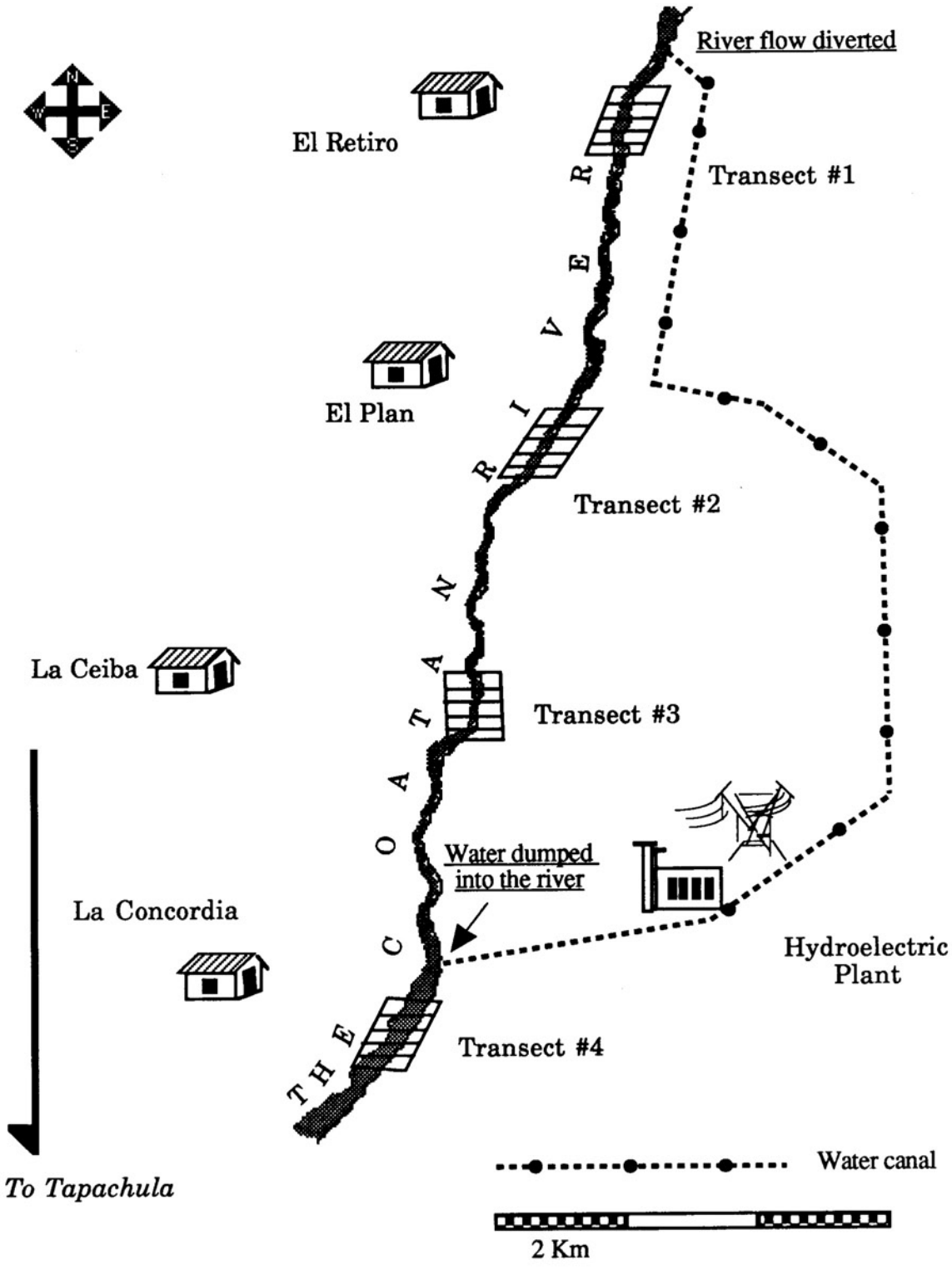

Figure 1. Location of transects along the Coatan River and points where the water flow is intercepted and later returned. The study area is located in the foothills near Tapachula, Mexico.

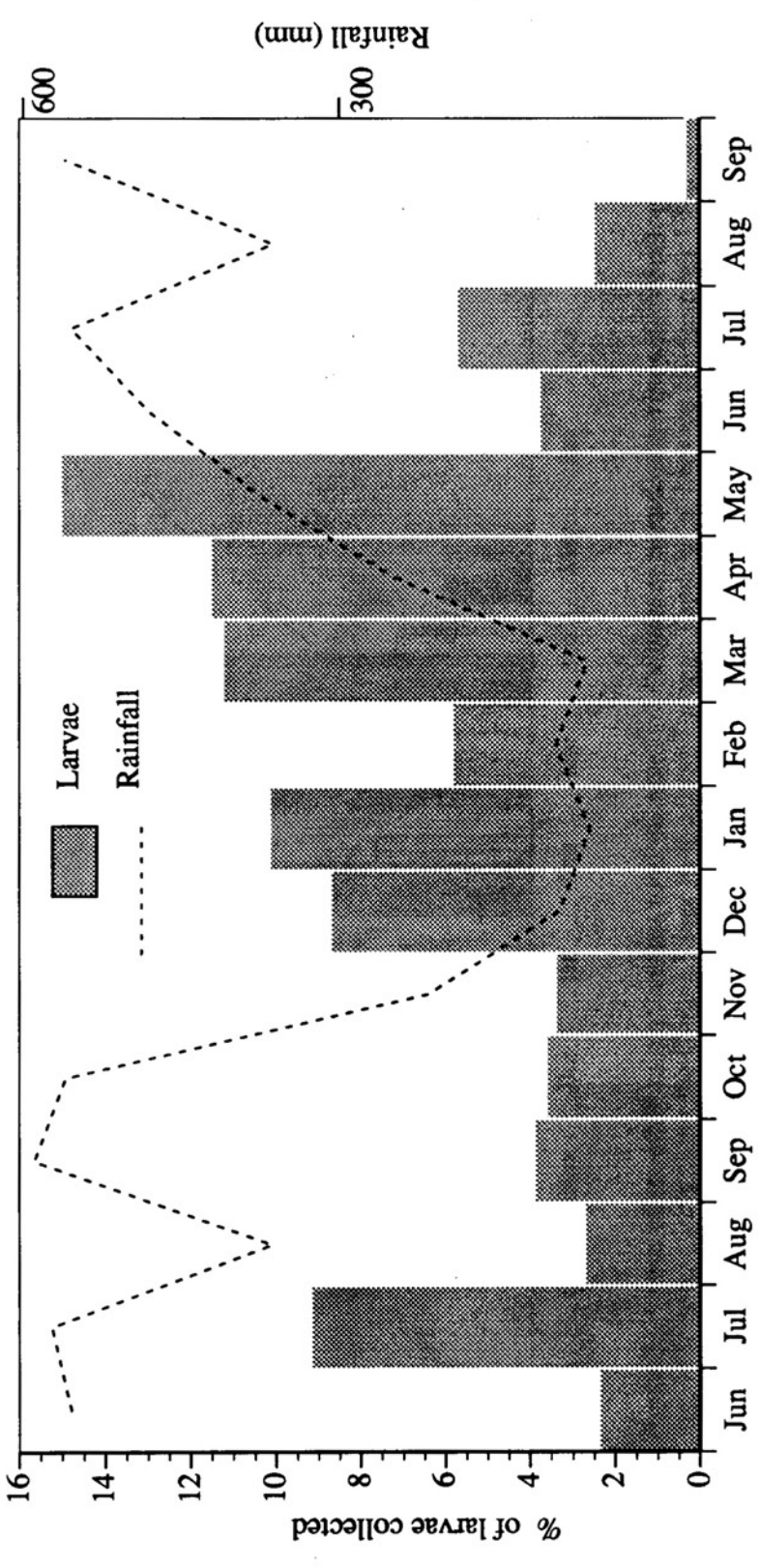

Figure 2. Abundance of *Anopheles pseudopunctipennis* larvae in wet season habitats versus amount of rainfall per month in the foothills near Tapachula, Mexico. Data are plotted by percentages per month based on total numbers collected from June 1990 to September 1991. Numbers of larvae were compiled by continuous monitoring of habitats that were characteristic of the wet season.

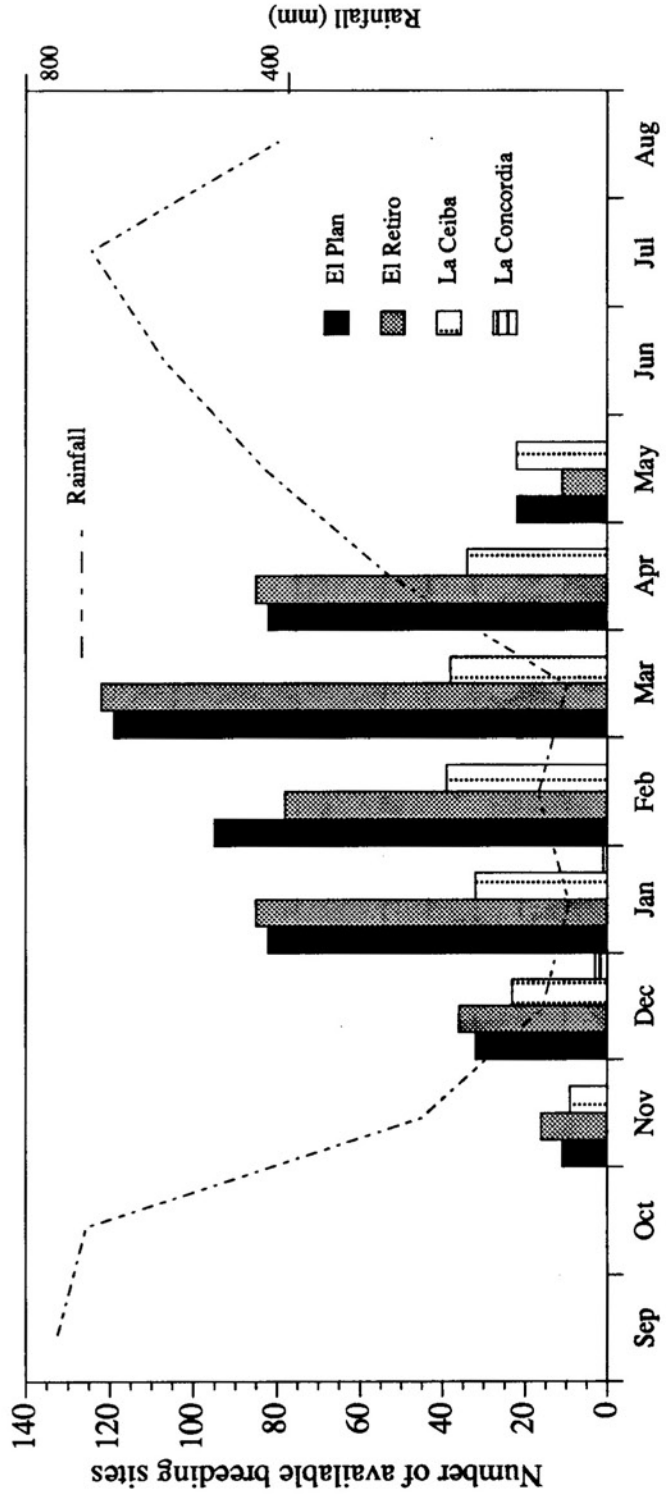

Figure 3. Pattern of monthly rainfall versus total numbers of *Anopheles pseudopunctipennis* breeding sites in four transects along the Coatan River in the foothills near Tapachula, Mexico (September 1990 to August 1991).

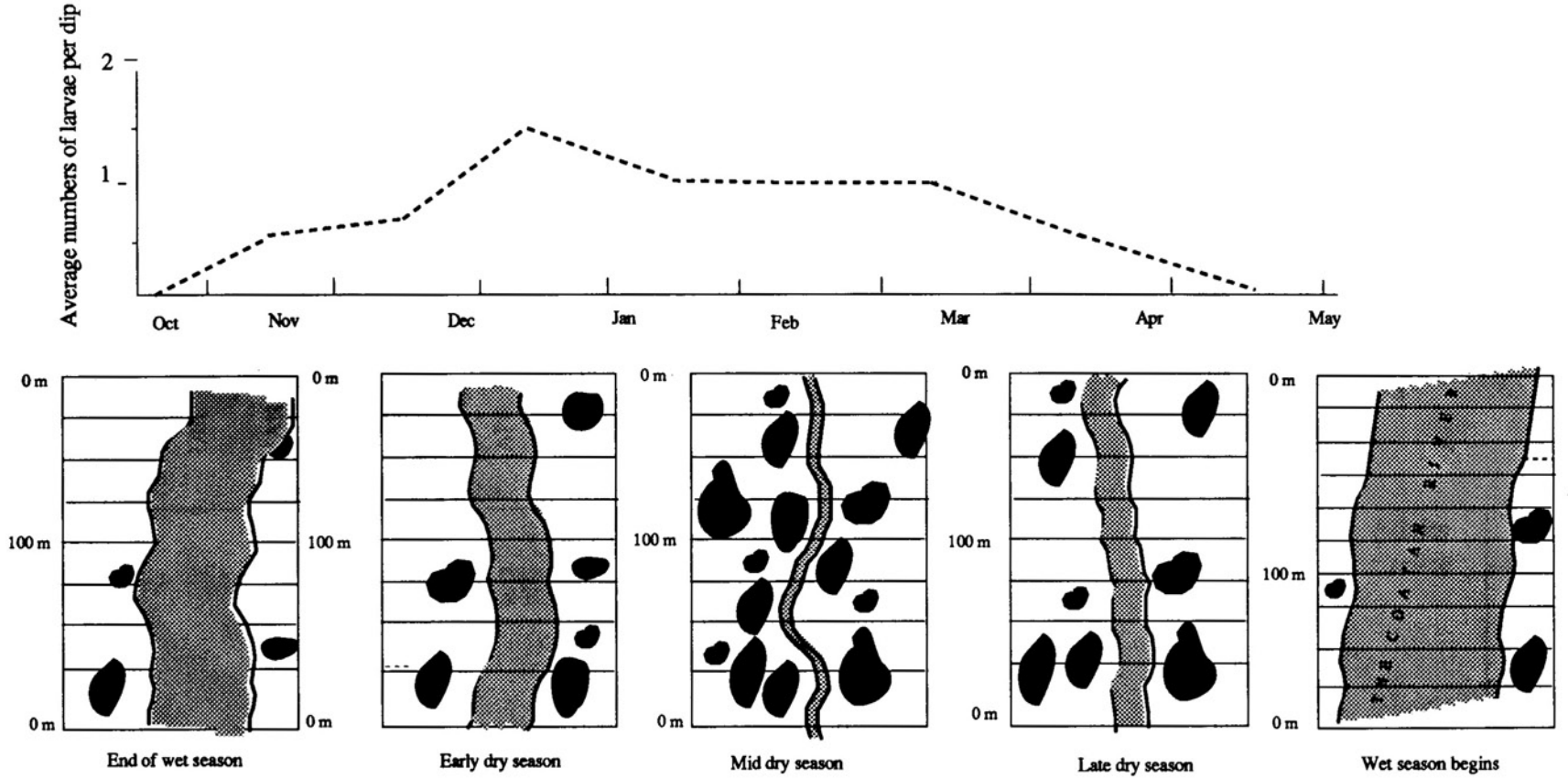

Figure 4. Seasonal changes in habitat availability as numbers of habitats for *Anopheles pseudopunctipennis* larvae and densities of larvae per habitat increases during mid-dry season, then decline with onset of rains in the wet season. Illustration is based on quantifying numbers of pools in four transects of the Coatan River in the foothills near Tapachula, Mexico (October 1990 to July 1991).

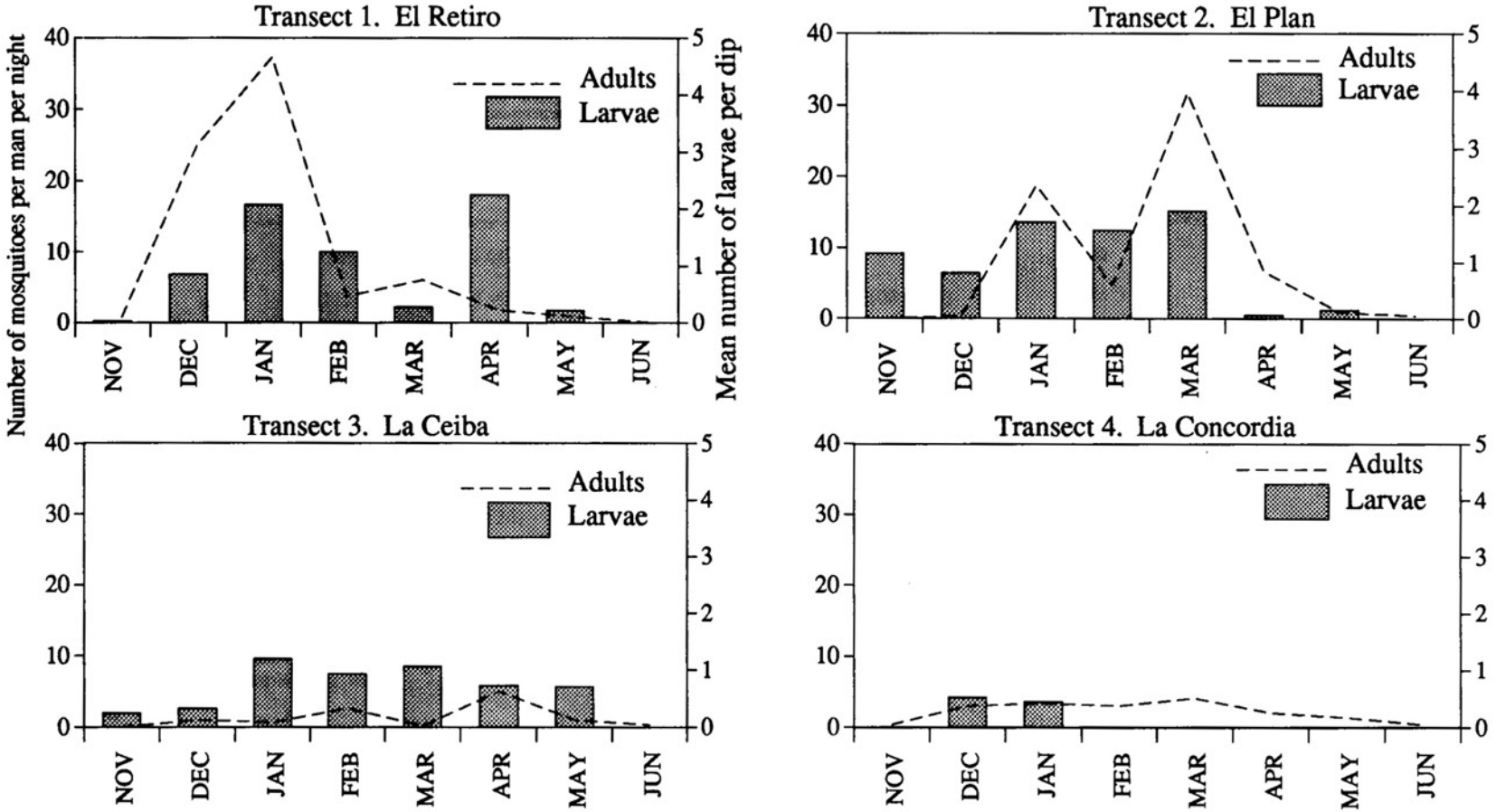

Figure 5. Monthly densities of *Anopheles pseudopunctipennis* larvae and adults at four sites along the Coatan River in the foothills near Tapachula, Mexico. (December 1990 to May 1991). Densities of adults were obtained by pooling data from all indoor and outdoor landing collections for each of 4 study villages (El Retiro, El Plan, La Ceiba and La Concordia). Densities of larvae are based on data from river transects at sites closest to each of the four villages.

Table 1. Habitat availability and relative numbers of *Anopheles pseudopunctipennis* larvae produced by different categories of aquatic habitats in the foothills near Tapachula, Mexico from June 1990 to September 1991.

Category of Habitat	*n*	% larvae by habitat	Number of visits[a]	% visits[b] positive for larvae	% visits habitats contained water (number) in the Wet Season[c]	% visits habitats contained water (numbers) in the Dry Season[d]	Mean number of larvae per dip in the Wer Season[f]	Mean number of larvae per dip in the Dry Season[g]
Springs	8	25.1	76	56.6 (43)	83.0 (63)	11.8 (9)	0.70	0.68
Ponds	9	21.9	141	30.5 (43)	98.0 (138)	100 (141)	0.48	0.79
Rain puddles	17	15.2	111	22.5 (25)	87.4 (97)	18.0 (20)	0.38	1.12
River margin pools	8	15.1	93	40.0 (37)	90.3 (84)	0.0 (0)	0.41	0.34
Streams	11	14.7	132	24.0 (31)	91.0 (120)	25.0 (33)	0.29	1.29
Lagoons	8	6.9	97	25.0 (24)	93.8 (91)	87.6 (85)	0.25	0.62
Gravel pits	3	0.5	38	13.2 (5)	97.4 (37)	65.7 (25)	0.12	0.20
Reservoir	1	0.5	16	25.0 (4)	100 (16)	100 (16)	0.12	4.66
Totals	*65*	*100*						

[a]Pooled monthly and fortnightly habitat sampling.

[b]Larvae were found

[c]Times habitat had water in Wet Season/Total sampling visits.

[d]Times habitat had water in Dry Season/Total sampling visits

[f]Total number of larvae /number of positive visits in the wet season/number of dips per visit (30).

[g]Total number of larvae /number of positive visits in the dryt season/number of dips per visit (30).

Table 2. Associations of *Anopheles pseudopunctipennis* larvae with plants in different categories of wet season habitats that were sampled in the foothills near Tapachula, Mexico from June 1990 to September 1991.

	Categories of wet season breeding sites								
Associated populations	Rain puddles	Streams	Ponds	Springs	Lagoons	River margin pools	Gravel pits	Reservoir	Totals
n	17 (26.2)†	11 (17.0)	9 (13.8)	8 (12.3)	8 (12.3)	8 (12.3)	3 (4.6)	1 (1.5)	65 (100)
An. pseudopunctipennis	8 (47.1)¥	10 (91.0)	7 (77.8)	8 (100)	7 (87.5)	7 (87.5)	3 (100)	1 (100)	51 (78.5)
Filamentous algae	6 (35.3)	8 (72.7)	4 (44.4)	8 (100)*	2 (25.0)	2 (25.0)	-	-	30 (46.2)
PLANTS									
Paspalum sp.	9 (53.0)	4 (36.4)	5 (55.6)	1 (12.5)	2 (25.0)	3 (37.5)	2 (66.7)	1 (100)	27 (41.5)
Ludwigia octovalvis	3 (17.6)	4 (36.4)	-	3 (37.5)	2 (25.0)	5 (62.5)*	-	-	17 (26.2)
Cyperus sp.	3 (17.6)	3 (27.3)	1 (11.1)	-	3 (37.5)	3 (37.5)	1 (33.3)	-	14 (21.5)
Heteranthera limosa	4 (23.5)	1 (9.1)	-	4 (50.0)*	-	-	-	-	9 (13.8)
Panicum sp.	1 (6.0)	-	1 (11.1)	-	1 (12.5)	3 (37.5)*	-	1 (100)	7 (10.8)
Commelina sp.	1 (6.0)	3 (27.3)	1 (11.1)	1 (12.5)	-	-	1 (33.3)	-	7 (10.8)
Mimosa sp.	3 (17.6)	-	-	-	-	-	3 (100)	-	6 (9.2)
Echinochloa colonum	2 (11.8)	-	-	2 (25.0)	1 (12.5)	1 (12.5)	-	-	6 (9.2)
Eichhornia crassipes	-	-	3 (33.3)*	-	1 (12.5)	-	-	-	4 (6.2)
Pistia stratiotes	-	-	3 (33.3)*	-	1 (12.5)	-	-	-	4 (6.2)
Oryza latifolia	-	1 (9.1)	-	-	2 (25.0)	-	-	-	3 (4.6)
Ipomoea sp.	-	-	-	1 (12.5)	1 (12.5)	-	-	-	2 (3.1)
Podostemaceae	-	-	-	-	2 (25.0)	-	-	-	2 (3.1)
Fimbristylis sp.	1 (6.0)	-	-	1 (12.5)	-	-	-	-	2 (3.1)
Veronica cf. peregrina	1 (6.0)	1 (9.1)	-	-	-	-	-	-	2 (3.1)
Cynodon dactylon	2 (11.8)	-	-	-	-	-	-	-	2 (3.1)
Dichronema sp.	1 (6.0)	-	1 (11.1)	-	-	-	-	-	2 (3.1)
Heliconia sp.	1 (6.0)	1 (9.1)	-	-	-	-	-	-	2 (3.1)
Pennisetum sp.	1 (6.0)	-	-	-	-	-	-	-	1 (1.5)
Bulbostylis sp.	-	1 (0.0)	-	-	-	-	-	-	1 (1.5)
Typha domingensis	-	-	1 (11.1)	-	-	-	-	-	1 (1.5)

†Number of sites and per cent ( ) of all available categories of breeding sites.
¥Number of positive sites (%)
* P<0.05, Fisher Exact Test, two tails.
- Not found.

Table 3. Numbers of habitats (river pools) and densities of *Anopheles pseudopunctipennis* larvae per habitat in each of four transects along the Coatan River in the foothills near Tapachula, Mexico. The dry season habitats within transects were sampled from November 1990 to June 1991.

Monthly Sampling	Transect 1 at El Retiro		Transect 2 at El Plan		Transect 3 at La Ceiba		Transect 4 at La Concordia	
	Number of habitats	Average number of larvae†	Number of habitats	Average number of larvae†	Number of habitats	Average number of larvae†	Number of habitats	Average number of larvae†
Nov	9	0.00	2	0.0	3	0.00	0	0.0
Nov	16	0.03	11	1.14	9	0.24	-	-
Dec	25	0.30	32	0.87	-	-	3	0.53
Dec	36	1.41	32	0.73	23	0.32	-	-
Jan	51	1.95	48	1.22	23	0.62	2	0.45
Jan	85	2.21	82	2.17	27	1.75	1	0.20
Feb	78	1.24	95	1.56	32	0.70	-	-
Feb	-§	-	-	-	39	1.15	0	0.00
Mar	90	0.28	122	1.74	35	1.06	0	0.00
Mar	122	2.13	119	2.04	38	1.11	0	0.00
Apr	121	2.37	95	0.12	38	0.28	0	0.00
Apr	85	0.02	82	0.01	34	0.11	-	-
May	11	0.40	22	0.16	22	0.01	0	0.00
Jun	0	0.00	0	0.00	0	0.0	0	0.00
Means	56.1	1.40	57.1	1.24	24.8	0.72	0.7	0.45

†Mean number of larvae per dip.
§Not sampled.

Table 4. Associations of *Anopheles pseudopunctipennis* larvae with habitats (river pools) containing filamentous algae in each of four transects along the Coatan River in the foothills near Tapachula, Mexico. Transects were sampled from November 1990 to June 1991.

Month Sampled	Number of habitats (pools)	% habitats with larvae	Avergae surface area of habitat (m^2)	% habitats with filamentous algae	Number larvae per m^2 of filamentous algae §
Transect 1 at El Retiro					
Nov	9	0.0	6.5	22.2	0.0
Nov	16	25.0	4.7	87.5	0.3
Dec	25	56.0	3.2	88.0	3.2
Dec	36	66.7	2.0	100	18.2
Jan	51	86.3	1.8	84.3	26.2
Jan	85	87.1	1.5	86.0	42.1
Feb	78	87.2	2.1	69.2	55.3
Mar	90	79.0	1.8	79.0	35.2
Mar	122	96.0	2.2	78.0	36.3
Apr	121	94.2	1.5	91.7	49.2
Apr	85	33.0	1.0	15.3	62.0
May	11	54.5	2.5	18.2	27.5
Jun	0	0.0	0.0	0	0.0
Means		*76.6*	*1.9*	*73.5*	*33.0*
Transect 2 at El Plan					
	2	0.0	2.5	50.0	0.0
	11	72.7	2.3	91.0	15.8
	32	75.0	0.6	100	14.7
	32	78.1	1.4	78.1	14.5
	48	87.5	1.1	79.2	16.0
	82	89.0	1.1	96.3	45.2
	95	88.4	0.8	89.5	54.2
	122	95.1	1.4	75.4	24.8
	119	82.4	1.1	93.3	46.8
	95	75.8	1.4	83.2	4.4
	82	12.2	1.8	47.6	10.0
	22	36.4	1.4	0.0	0.0
	0	0.0	0.0	0.0	0.0
		75.5	*1.2*	*79.6*	*28.6*
Transect 3 at La Ceiba					
Nov	3	0.0	0.9	33.3	0.0
Nov	9	33.3	1.7	77.8	3.3
Dec	-†	-	-	-	-
Dec	23	52.2	1.5	87.0	5.4
Jan	23	56.5	1.4	91.3	11.5
Jan	27	85.2	1.1	85.2	33.4
Feb	32	68.8	0.6	84.4	13.6
Feb	39	89.7	1.0	69.2	21.7
Mar	35	85.7	1.3	63.0	13.2
Mar	38	86.8	1.1	71.1	21.4
Apr	38	55.3	0.8	55.3	22.7
Apr	34	44.1	0.8	17.6	27.4
May	22	4.5	1.0	0.0	0.0
Jun	0	0.0	0.0	0.0	0.0
Means		*64.4*	*1.1*	*62.5*	*16.7*
Transect 4 at La Concordia					
	0	0.0	0.0	0.0	0.0
	-	-	-	-	-
	3	66.7	0.2	100	43.7
	-	-	-	-	-
	2	50.0	0.1	100	66.7
	1	100	0.2	0.0	0.0
	0	0.0	0.0	0.0	0.0
	0	0.0	0.0	0.0	0.0
	0	0.0	0.0	0.0	0.0
	-	-	-	-	-
	0	0.0	0.0	0.0	0.0
	-	-	-	-	-
	0	0.0	0.0	0.0	0.0
	0	0.0	0.0	0.0	0.0
		66.7	*0.2*	*83.3*	*53.9*

§Total number of larvae/total cumulative area (m^2) of all habitats.
†Not sampled.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS AND RECOMMENDATIONS

This research has contributed new information on the biology of *An. pseudopunctipennis* and new insights into its role as a vector of malaria in southern Mexico.

In the following discussion, components of the vector life cycle that are traditionally employed in defining vectorial capacity will be emphasized. This discussion will also include observations on other population parameters which will contribute to a better understanding of the mosquitoes' capabilities to transmit malaria.

The estimate of vectorial capacity constitutes an attempt to quantify an important component of malaria epidemiology. Vectorial capacity has been defined by Garret-Jones and Shidrawi (1964) as "the average number of inoculations with a specified parasite, originating from one case of malaria in unit of time, that a vector population would distribute to man if all the vector females becomes infected." It is also a convenient way to express malaria risk, or in other words, the "receptivity" of a defined area to malaria (Bruce-Chwatt, 1985). The formula for vectorial capacity and its mathematical components are:

$$C = \frac{m\, a^2\, p^n}{-\log_e p} = \text{Vectorial Capacity}$$

m = Man-biting rate or number of mosquitoes/man/night.

a = Man-biting habit; this is the average frequency of biting on man by the individual mosquito, which also depends on the duration of the vector's gonotrophic cycle and the Human Blood Index (*HBI). It is calculated as the reciprocal of the numbers of days required for the gonotrophic cycle multiplied by the HBI:

$$a = \frac{1}{G\ C} \times \text{HBI}$$

p = Probability of the female mosquito's surviving one day.

p^n = Probability of the female mosquito's surviving for *n* days (*n* = duration of the extrinsic incubation period; normally for *P. vivax* it is nine days, and 10-12 days for *P. falciparum*).

$\frac{1}{-\log_e p} \cdot a$ = Probability of infective female life, or the proportion of the populations surviving beyond the extrinsic incubation period, and biting another person "a" times per day.

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* HBI is defined as the proportion of females found to contain human blood.

Comparative statistics on vectorial capacities have been calculated for *An. pseudopunctipennis* in the Tapachula foothills for the dry seasons of 1990 and 1991, and for the four villages, two with low (La Ceiba and La Concordia) and two with high (El Plan and El Retiro) vector densities (Table 1 and 2). Data from months with higher vector densities were used in these calculations to show the density-dependent relationships of vectorial capacity estimates. The tabular data were derived from indoor host-seeking collections that were reported in chapters 2, 3 and 4.

Table 1. Vectorial capacity (C) and other parameters used to calculate vectorial capacity of *Anopheles pseudopunctipennis* mosquitoes in each of four study villages in the foothills near Tapachula, Mexico. Data on natural populations were collected during January and February 1990.

Expression	El Retiro	El Plan	La Ceiba	La Concordia
	January	February	January	February
ma *	27	23	4	9
G C	3	3	3	3
HBI**	0.36	0.37	0.06¥	0.06
a	0.12	0.12	0.02	0.02
PR*	0.68	0.40	0.85	0.33
p†	0.8793	0.7368	0.9472	0.6910
n	9	9	9	9
p^n	0.2763	0.0639	0.6137	0.0359
$1/-\log_e p$	7.7 days	3.2 days	18.4 days	2.7 days
C	7.8	0.6	0.9	0.2

GC= Number of days required for completing the gonotrophic cycle; HBI= Human Blood Index; PR= Parous rates; C= Vectorial Capacity.
*Numbers landing on a human host indoors
**Unweigthed HBI
† Computed according to Davidson's formula (1954).
¥Standarized with La Concordia value because no blood-engorged specimens were collected in 1990.

Table 2. Vectorial capacity (C) and other parameters used to calculate vectorial capacity of *Anopheles pseudopunctipennis* mosquitoes in each of four study villages in the foothills near Tapachula, Mexico. Data on natural populations were collected during December 1990 to March 1991.

Expression	El Retiro	El Plan	La Ceiba	La Concordia
	December	January	March	December
ma *	18	20	5	3
G C	3	3	3	3
HBI**	0.42	0.38	0.11	0.05
a	0.14	0.13	0.04	0.02
PR*	0.450	0.46	0.47	0.50
p†	0.7663	0.7719	0.7774	0.7937
n	9	9	9	9
p^n	0.0911	0.0972	0.1037	0.1249
$1/-\log_e p$	3.7 days	3.8 days	3.9	4.3 days
C	0.8	0.9	0.08	0.03

C= Number of days required for completing the gonotrophic cycle, HBI= Human Blood Index, PR= Parous rates, C= Vectorial Capacity.
*Numbers landing on a human host indoors
**Unweigthed HBI
† Computed according to Davidson's formula (1954).

Large numbers of malaria cases were recorded in 1990 and 1991 for the villages of El Retiro and El Plan. In contrast, malaria cases were relatively uncommon in the villages of La Concordia and La Ceiba (Loyola, E. G., 1991, personal communication).

Estimates of vectorial capacity (C) as high as 7.8 (El Retiro, 1990) and as low as 0.03 (La Concordia, 1991) were computed for *An. pseudopunctipennis* populations in the study villages (Tables 1 and 2). Higher estimates of vectorial capacity were calculated for populations associated with El Retiro (7.8) and La Ceiba (0.9) in 1990. The high vectorial capacity in a village with few malaria cases (La Ceiba) can probably be explained by the low densities of vector populations (4 m/m/n). Vector capacity for *An. pseudopunctipennis* populations in El Plan was only 0.6, but more cases were recorded in this village than for either La Ceiba or La Concordia. High vector population densities (23

m/m/n) and HBI (37%) probably favored the higher malaria infection rates in the vectors (*P. vivax* CS-protein rate of 0.001, or 1/859), and larger numbers of malaria cases than expected from the estimates of vectorial capacity alone. Alternatively, the higher estimate of vectorial capacity (0.9) of populations at La Ceiba was probably due to high vector survival rates. At this point, we need to emphasize the importance of survival estimates as denominators in these calculations. As stated by Garret-Jones and Shidrawi (1969), an error in calculating daily survival rates will have drastic consequences in the final estimates of vectorial capacity.

There was general agreement in the estimates of vectorial capacity and the numbers of malaria cases during the dry season of 1991 (Table 2). El Retiro and El Plan had higher malaria incidence and vectorial capacity rates, while the opposite was seen in La Ceiba and La Concordia (Loyola, 1991, personal communication). Mosquitoes caught at La Ceiba in 1991 were physiologically younger than specimens collected in 1990 (PR 0.85 versus 0.47). Consequently, the expectancy of infective life was only 3.9 days in 1991, compared to 18.4 days in 1990. Chemical control measures applied to these villages probably accounted for the overall reduction in vector longevity, and this effect had a direct impact on estimates of daily survival. Accordingly, as stated by Bruce-Chwatt (1985), vector control by larvicides or other measures will affect *m*, screening of houses will reduce a, while residual spraying will greatly reduce the factor *p*.

Based on the estimates of vectorial capacity, *An. pseudopunctipennis* mosquitoes appear to be highly efficient vectors of malaria under conditions encountered in foothill areas of southern Mexico. A better idea of *An. pseudopunctipennis* vectorial efficiency is obtained by comparing the estimates of vectorial capacity of this species with estimates for *An. albimanus* and *An. vestitipennis* (Table 3). *Anopheles albimanus* is the more important vector in the coastal plains of Mexico and Central America, whereas, *An. vestitipennis* is a local vector in areas such as the Lacandon forest in Chiapas (Loyola, *et al.*, 1991), and in localities in other states in Mexico, as well as in Guatemala and the Caribbean (Pan American Health Organization, 1991). The comparative data were derived from studies by Mekuria, *et al.* (1991) conducted in the Dominican Republic.

Table 3. Vectorial capacity estimates of malaria vectors from Dominican Republic and southern Mexico.

Expression	*An. albimanus*	*An. vestitipennis*	*An. pseudopunctipennis* Highest VC¥	*An. pseudopunctipennis* Lowest VC†
ma *	15.7	13.8	27	3
G C	2.6	3.2	3	3
HBI**	0.08	0.12	0.36	0.05
a	0.031	0.038	0.12	0.02
PR*	0.373	0.207	0.68	0.50
p††	0.684	0.611	0.8793	0.7937
n	11*	11	9	9
p^n	0.015	0.005	0.3142	0.0992
$1/-\log_e p$	2.6 days	2.0 days	7.7 days	4.3 days
C	0.019	0.005	7.8	0.03

¥El Retiro 1990, Table 1
†La Concordia 1991, Table 2
*Extrinsic incubation period for *P. falciparum*.

As seen in Table 3, even the lowest estimate of vectorial capacity of *An. pseudopunctipennis* (0.03) is slightly higher than the estimated vectorial capacity for *An. albimanus* (0.019) and much higher than the estimated value for *An. vestitipennis* (0.005) in the Dominican Republic. A comparison of individual components of the vectorial capacity in Table 3 reveals two features that result in higher values for *An. pseudopunctipennis; viz.*, a higher HBI and greater longevity as determined by the parous rate (PR). The Human Blood Index indicates that *An. pseudopunctipennis* more readily feeds on humans than the other two anophelines. As discussed in Chapter 3, *An. pseudopunctipennis* is known throughout its geographical range as an anthropophagic mosquito. Additionally, the high parous rates for *An. pseudopunctipennis* result in higher estimates of daily survival (*p*), which increases the estimate of vectorial capacity.

There are other biological characteristics of *An. pseudopunctipennis* that contribute to its role as an important malaria vector. These factors are not included in the classical model of vectorial capacity. One such factor comes from our observation that older females seem to feed later at night (see Chapter 4). This behavior increases the

probability of infectious females getting a complete blood meal and transmitting malaria while the human host is asleep. Increased feeding success would increase *ma*, HBI and a. Additionally, about 25% of the host-seeking *An. pseudopunctipennis* are pre-gravids. Pre-gravid females require a second blood meal in order to complete egg development. The pre-gravid phenomenon facilitates the process of malaria transmission. As an example, if the probability of a mosquito's feeding on a human host during one feeding event is 0.3, then the probability of its taking two human blood meals, during two sequential feeding events is 0.09 (0.3 X 0.3). However, it is important that the mosquito take an infectious human bloodmeal as soon as possible in order to acquire the malaria infection, survive long enough to complete extrinsic incubation, bite another human and transmit malaria. Given this concern, the probabilities of its taking at least one meal from a human host during two separate but sequential feeding events is 0.6 (0.3 + 0.3). Clearly, the pre-gravid phenomenon results in an increased man-biting rate, *ma*, and also HBI. Moreover, this gonotrophic discordance may mislead interpretations of numbers of blood meals by standard age-grading techniques, *i.e.*, two dilatations may indicate three blood meals, not two.

Knowledge of some other characteristics of *An. pseudopunctipennis* populations may serve to improve control strategies for *An. pseudopuntipennis*-transmitted malaria in Mexico. The greatest human-vector contact was found to occur in those villages (El Retiro and EL Plan) that were closest to the Coatan River (see Chapter 4). In addition to the importance of village location in relationship to vector breeding sites, the status of the chemical control measures also influence the degree of human-vector contact. As the insecticide residues on housewalls degraded, the levels of anthropophily and endophily increased. This effect seemed to occur about three months after houses were sprayed with DDT (see Chapter 3).

The indoor host-seeking and resting behavior of *An. pseudopunctipennis* would seem to favor the use of house spraying with DDT or Bendiocarb as a method of vector control. Indeed, the numbers of specimens feeding and resting inside of houses dropped dramatically after houses were sprayed with DDT (chapter 3 and 4). Further studies on physiological and ethological resistance of *An. pseudopunctipennis* to DDT and other insecticides should be performed to define efficacy and mode of action in malaria control.

The results of chemical control measures are strikingly increased if properly timed. For example, a high degree of vector control was achieved in 1991 when houses were sprayed at the beginning of the dry season. This is in contrast to the negligible effect of spraying houses at the end of the dry season, as was done in 1990. Obviously, house

spraying at the end of the dry season will coincide with the disappearance of breeding sites and a natural decline in vector density.

Considering the dramatic effect of chemical control on vector populations in the study villages, properly timed and executed house-spraying may eliminate malaria from some localities in the future. However, long-term measures, such as larval control by water level management, should also be implemented. In spite of a relatively high initial cost, environmental management is cost-effective on account of long-term efficacy, low maintenance cost and safety (Rafatjah, 1988). Population density of *An. pseudopunctipennis* is greatest in the dry season as breeding sites become available in rivers and streams. In the case of the four study villages, most anophelines were produced in breeding sites in the Coatan River. Furthermore, there were relatively few alternative breeding sites around these villages during the dry season (Chapter 5). As with pools in the Coatan River, the few alternative breeding sites were easy to locate and treat with larvicides.

Water in the Coatan River is diverted above El Retiro and is moved by canal to a hydroelectric plant. The water re-enters the Coatan River below La Ceiba and above the village of La Concordia. The segment of river between El Retiro and La Ceiba, with greatly reduced water flow, produces the greatest densities of *An. pseudopunctipennis* larvae. This relationship was described in chapter 5. Based on observations made during this study, the existing Coatan River hydrological system seems amenable to water level management as a means of vector control. Evidence for this was obtained in March 1991 when normal water flow was temporarily returned to the river while the canal was repaired and maintained. The temporary increase in water flow flushed the mats of filamentous algae from the river bed and anopheline production decreased (Fig. 1, Chapter 5). Use of water level management could be employed by filling the reservoir before the end of the wet season (November) and by flushing the breeding sites beginning in December. Sites would probably need to be flushed at weekly intervals during the peak breeding season (January-February). The actual numbers of hours of increased water flow necessary to flush and destroy breeding sites are unknown. This schedule of water management could be terminated in May of each year.

Given the focal characteristics of malaria transmission, this research has provided new information on the ecology of *An. pseudopunctipennis* and malaria ecology in the foothill region near Tapachula, Mexico. Nevertheless, much research is needed to better define the role of this vector species in transmitting malaria in other countries and other states in Mexico. In addition to needing studies on vector responses to insecticides, as mentioned previously, more research is needed to quantify vector susceptibility to the

malarial parasites, particularly the two strains of *P. vivax*. Furthermore, studies employing direct examinations of salivary glands for sporozoites would be useful for validating the results of ELISAs for detecting *P. vivax* CS-proteins. Further investigations on the role of multiple feedings by pre-gravid populations should be conducted to evaluate the contribution of this phenomenon to the transmission of malarial parasites. Many of the studies, as described, could be greatly facilitated by success in developing a self-mating colony of *An. pseudopunctipennis*.

In conclusion, this research has provided a clear indication of the overall importance of entomological research in advancing our understanding of malaria epidemiology. We agree with Garret-Jones (1970) that epidemiological entomology should be part of integrated malaria research everywhere the disease is present. Garret-Jones and Shidrawi (1969) defined epidemiological entomology as "the sampling and study of vector populations in order to assess, according to the range of vector prevalence, longevity, biting-rhythm, host selection, *etc.*, the expected trends in the incidence of transfer of a pathogen to a vertebrate host and back to the vector itself, whether before, during or after the operation of control measures." This definition emphasizes the importance of epidemiological factors versus those of merely ecological importance. As stated by Garret-Jones and Shidrawi (1969), observations on parameters of epidemiological entomology should receive high priority, as these parameters are the measurements required to place the entomologist in a position to advise the public health authorities as to the planning, assessment, duration and supplementation or modification of the attack measures directed at the vectors of malaria.

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ANNEXES 1 TO 9

ANNEX 1

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